COLLOID SYMPOSIUM MONOGRAPH

PAPERS PRESENTED AT THE
SECOND NATIONAL SYMPOSIUM ON COLLOID CHEMISTRY
NORTHWESTERN UNIVERSITY, JUNE, 1924

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FOREWORD

This monograph presents the intellectual output of the Second Annual Colloid Symposium held at Northwestern University in June, 1924. The twenty papers here produced cover a wide field and no colloid chemist can afford to do without them.

The monumental paper by Dr. R. A. Gortner and his associates at Minnesota represents years of research and is a masterly reply to certain implied criticisms of colloid chemistry found in Loeb's book on Proteins and Colloid Behavior. We enjoy the privilege of giving half the space in this book to such a paper.

We are fortunate, too, in having among our valued contributors such men as Dr. Freundlich of Germany, Dr. Michaelis of Japan and Dr. Whitby of Canada. This gives the desired international flavor.

The range of subjects is interesting. Clays, rubber, emulsions, distribution of particles, plasticity and ion effects are among the topics.

In 1925 the Symposium will be held at the University of Minnesota and the papers read will be collected in a book similar to this. A tradition is being built up.

Each symposium is sponsored by the Colloid Committee of the National Research Council.

Harry N. Holmes.

Oberlin College,
Oberlin, Ohio.

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The following papers were read at the Second National Symposium on Colloid Chemistry held at Northwestern University, June 18th to 21st, 1924.

COLLOID SYMPOSIUM MONOGRAPH

GENERAL PRINCIPLES OF THE EFFECTS OF IONS IN COLLOIDS

By Leonor Michaelis

It is generally known that the conditions of colloids are determined by electrolytes added to the colloidal solutions and that as a rule we may clearly discriminate the effect of the single ions which are the components of the electrolytes. It is true, there are other methods for altering the colloidal conditions, too, such as change in temperature, change of the solvent medium, in a certain degree even addition of non-electrolytes. However, among these influences no other has exerted such an attractive power on the mind of colloid chemists as the influence of ions. Nevertheless a general theory concerning the effects of ions has not yet been developed. The reason is certainly that a general uniform theory is not possible at all. The mechanism of ion effects may be quite different in different cases. Though the elucidation of all of these ion effects is not yet possible to-day, yet it seems to me that the time has come to make an attempt to a general and systemical consideration. The supply in experimental and theoretical work might be sufficient. In all events such an attempt reveals the deficiencies of knowledge and may stimulate by arousing agreement or contradiction.

In my opinion all effects of ions may be classified in two groups, which are essentially different from each other. One might call them the *direct* effect and the *indirect* effect of ions.

The direct effect is based on the fact that an ion can be adsorbed by a colloidal particle. Here I am availing myself of the term adsorption only in the meaning, that the ion is fixed or attached at the superficies of the colloidal particle, no statement being made whether the force of this adsorption is a physical or a chemical force. I wish to emphasize that in my present paper "adsorption" does not mean a contradistinction to "chemical combination." The fact which is concerned is only the fixation of an ion on the colloidal micella. Now, the electrical charge of such an ion will exert certain electrical effects. This effect may be called the direct or the electrostatic effects of the ions. This effect is based on the free electrical charges of the ions.

On the other hand when two substances are dissolved in a common

solvent medium, they exert an influence one on another. This influence may be very small in the case of dissolved unelectrical molecules of non-electrolytes, but there is as a rule a considerable effect in the case of a mixture of two electrolytes. For instance the solubility of one electrolyte may be strongly altered by the presence of another electrolyte. Just so the colloidal condition of the micellae may be influenced by an ion which is dissolved in the same solvent medium without this ion being fixed on the colloid and without, perhaps, the micellæ carrying any adsorbed ions which could impart an electrical charge to the micellæ. The effect of these ions takes place at a distance. However since we commonly reduce such influences at a distance to an intermediary effect of the medium, we may assume that the solvent medium, mostly the water, is the intermediary medium for this effect. We may therefore interpret this kind of effect as a competition for the water, of the dissolved ions on the one hand, and of the micellae on the other hand. Such a competition can only take place in such cases in which the micellæ have an affinity for the water, comparable to the affinity of a dissolved substance to the water. These colloids are the lyophilic colloids, which form a stable colloidal solution even at their isoelectric point, while the hydrophobic colloids have no affinity for the water and form a stable suspension only when protected by the electrical charges of the adsorbed ions. We may designate the effect of ions the *indirect* or the *hydrophilic* effect.

Now let us begin with the discussion of the direct effect of the ions. When a certain number of ions of homonymous electrical charge are attached at the superficics of a micella, electrostatic forces cause a layer of ions of opposite sign to accumulate in the surroundings, and an electrical double layer is formed, the effect of which is cataphoresis, endosmosis, stability of the colloid and other well known phenomena. The main question is to find out the cause which brings about this electrical double layer. The primary evenement is always the adsorption of the one kind of ion. The formation of the other layer with opposite sign is a purely electrostatic consequence. What are the causes which bring about the adsorption of ions?

It seems to me there are three possibilities which might produce the formation of a double layer. I will provisionably designate them by three short terms: Firstly, double layers in consequence of appositional adsorption; secondly, double layers in consequence of the tendency of dissociation in colloidal particles, and thirdly, double layers produced without active chemical participation of the material of the boundary surface. I begin with

Double layers in consequence of appositional adsorption. LOTTERMOSER found the following fact: when a precipitate is produced by mixing a solution of silver nitrate and potassium iodide the produced silver iodide is positively charged when an excess of silver nitrate is

in the solution: it is negatively charged when there is an excess of potassium iodide. The reason for that may be easiest conceived according to an idea of HABER. The crystal grating of silver iodide is of that cubical order known to you, in which each valency is dissipated into six directions, so you may realize that the silver ions of the crystal, as far as they are situated in the surface of the crystal, have a partial valency free and directed outward, and likewise the iodine ions of the crystal. In virtue of this superficial residual valency a superficially situated silver ion of the crystal attracts an iodine ion of the solution, and an iodine ion of the crystal attracts a silver ion of the solution. According to the concentration of the silver or iodine ions in the solution the crystal should adsorb either silver or iodine ions. Such an adsorption may be called appositional adsorption, and there is no reason why this should only take place in crystals and not as well also in amorphous surfaces. Supposing such a surface is covered with free silver ions, in consequence of electrostatic forces a layer of negative ions must be formed in a certain distance from it. The ion adsorbed in higher degree on the given conditions determines the sign of the charge of the solid surface, while the ion inferior in adsorption determines the sign of the charge of the other layer. This other layer is situated in the fluid part not strongly adherent to the solid wall. It need not necessarily represent a proper surface in the mathematical sense, but the fact is better expressed by admitting the concentration of iodine ions in the neighborhood of the boundary layer to be larger than generally in the solution. To this idea which was pointed out by GOUY we shall return a little later on, as it is valuable for all kinds of double layers.

We shall now proceed to the second form of double layers, those in consequence of the dissociation tendency of a colloid. Here also let us illustrate the matter by an example. There are many chemical substances which according to their chemical character may be called heteropolar and even electrolytes, and which would behave like common electrolytes, *if they were soluble in water*. For instance, resin acids as mastic; nucleinic acids, silicic acid, and according to the excellent investigations of McBain we may add the higher aliphatic acids and the soaps. The sodium salts of the higher aliphatic acids are not dispersed to single molecules or ions, but form micellae, the interior of which contains fatty acid and sodium. The high conductivity for the electric current proves, however, some ions to be present. Hence we derive the following conception. The micellæ of a colloidal mastic solution, as well as any acid, have the tendency to dissociate into hydrogen ions and acid anions. However, the anions of this acid not being dispersible up to separated molecules, stay at the surface of the micella. We have, according to the idea of LANGMUIR, to imagine these anions to be elongated molecules, the negatively charged end of

which are directed towards the surface of the water, the other end is fixed at the micella. On the contrary, hydrogen ions are free and gather in the surroundings in consequence of the electrical charge of the anion layer, thus forming the exterior layer. As above, we need not suppose that they form a real surface but that they may be dispersed in a certain volumetric dimension. This holds for all double layers, as GOUY emphasized. GOUY called this condition of the double layer its *diffusity*. Therefoie colloid acids, as common acids, suspended in pure water, increase the *quantity* of the hydrogen ions but they do not increase the concentration of hydrogen ions at any place within the interior of the solution. They only bring about an accumulation of hydrogen ions round the micellae.

Supposing such a colloidal acid is not dissolved in pure water but in a watery solution of electrolytes, according to the conditions an exchange of hydrogen ions and other metal ions may take place, and this process is analogous to the formation of a salt in common acids.

We may call a colloidal substance which chemically owns the character of an acid yet produces no discrete but only colloidal ions, an *acidoid* in opposition to a truly soluble *acid*. When we bring such an acidoid into aqueous solutions of varied hydrogen ion concentration, avoiding ions as easily adsorbable as calcium or aluminum to be present, we may suppose the exterior layer to be formed only by hydrogen ions and the potential to depend only on the hydrogen ion concentration of this solution. This phenomenon has an appearance very similar to a metallic platinum-hydrogen-electrode, the potential of which also only depends on the concentration of hydrogen ions. Many years ago I emphasized this analogy for the first time, comparing the micellae of a colloidal acid to a hydrogen electrode. I then uttered the conjecture that the potential of such an electrode might depend on the hydrogen ion concentration according to the same logarithmic rule as the potential of a metallic electrode. This supposition has not been experimentally confirmed. While according to this law one should have expected that a variation of hydrogen ion concentration in the proportion that one is to ten, should produce a variation of 58 millivolts, this variation is really much smaller and secondly not constant for each power of 10 of hydrogen ion concentration. On the annexed diagram you see the reproduction of an experiment of mine, in which the potential of mastic particles is represented as a function of pH of a diluted acetate buffer. As you recognize that the change of potential of the mastic particles for a power of 10 in hydrogen ion concentration varies between about 4 to 11 millivolt, nowhere at any rate approaching up to 58 millivolts for this value. Appealing to the empirical fact, that mastic cannot be charged positively by any ever so high an acid concentration, we may complete the curve to the left side, as this left side is no more accessible for cataphorsis experiments on

account of flocculation. Evidently the potential asymptotically approaches to zero with increasing hydrogen ion concentration. This fact is strictly contradictory to NERNST'S logarithmic law in metallic electrodes. Well, I think the analogy to a hydrogen electrode was a fundamental mistake of mine, the explanation of which seems to me very important to the further development of the theory. Therefore, I wish to dwell somewhat on this subject.

In a metallic hydrogen electrode, following NERNST, we may admit two quantities of opposite sign; the hydrogen has a tendency to emit positively charged hydrogen ions into the solution, the electrode then remaining *negatively* charged. On the other hand the hydrogen ions of the aqueous solution have a tendency to settle into the metallic surface, the metal then being *positively* charged. According to the concentration of hydrogen ions the one tendency or the other prevails, and the electrode is sometimes positively charged, and sometimes negatively. The absolute zero point of charge is given approximately in a pH equal to 4.5, calculating from the absolute value of the calomel electrode. Thus, there is a definite pH in which the charge turns and which we might call the isoelectric point of the electrode. In a colloidal acidoid the conditions are quite different. It is true, an acidoid has the tendency to emit hydrogen ions, too, and therefore to remain negatively charged; but the hydrogen ions of the solution have not any tendency to settle into the surface of the solid particle so as to charge it positively. At the most, a number of hydrogen ions corresponding to the superficially situated anions of the solid particle may fix themselves to the surface, the charge then being equal to zero. More hydrogen ions are not able to stay at the surface, and no positive charge of the surface can arise. At the best, discharge takes place, when the hydrogen ion concentration is very high, similarly to the case of a true weak acid, the dissociation of which may be suppressed by the presence of a strong acid. Now, mastic and other acidoids are positively charged, though not by hydrogen ions yet by trivalent metal ions as aluminum. We shall return to this problem a little later.

However, there are also colloidal substances the charge of which is sometimes positive and sometimes negative according to the hydrogen ion concentration in the solution, as albuminoid substances, gelatin, casein and many others. They have an isoelectric point at a definite hydrogen ion concentration characteristic for each kind of protein. In this case, the similarity to a metallic electrode is more evident, and the reason is, that these colloids really have the tendency both to emit and to fix hydrogen ions, since they contain both carboxyl groups and amino groups. The carboxyl group may emit a hydrogen ion and thus remain positively charged. The amino group may combine with a hydrogen ion, forming an ammonium ion positively charged, according to the formula $R.NH_2 + H^+ = RNH_3^+$.

On the other hand $R.OOOH = R\,COO^- + H^+$.

These colloids behave like amphoteric electrolytes, and we may call them ampholytoids. We shall return once more to double layers of this kind and we shall now proceed to the third group, viz. to *the double layers formed without any active participation of the colloidal phase.* Neither of the possibilities just discussed is sufficient to explain all double layers that may occur; they only suffice in the case of such colloids, the micellae of which consist of a heteropolar electrolyte-like chemical compound. However, experience has taught that at the boundary surfaces of materials chemically wholly indifferent and not dissociated at all, generally an electrical double layer is also formed. To give some examples, the surfaces of cellulose, paper, collodion, agar, parchment paper, even gas bubbles, are negatively charged against aqueous and even sometimes non-aqueous solutions. It is striking that the aqueous phase is almost throughout negatively charged. COEHN pointed out a rule according to which each phase of a higher dielectric constant is negatively charged against another phase of a lower one. Quite generally this rule is certainly not fully suitable, for in this case there could not exist any substance positively charged against water, the dielectric constant of which is extremely high; yet there are plenty. A little more suitable becomes this rule when restricted to substances not leading to the formation of ions. Yet we are too little informed about the charges of boundary surfaces of phases not containing water at all, so let us confine ourselves to the following assertion. Any substance of electrochemically indifferent character is negatively charged against aqueous solutions or against phases containing some water. Positive charges occur extremely seldom even under such conditions, where electrolyte-like phases of generally negative charge change the charge. On this occasion, let us once more return to the problem of change of the sign of charge in the case of an acidoid. First remember that all these acidoids do not change the sign of the charge by hydrogen ion concentrations, however high. However, when trivalent or quadrivalent metal ions are present, they do change the sign of charge. For instance glass, silicates such as kaolin, mastic acquire a positive charge by aluminium salts in the lowest concentration. I wish your attention to be especially drawn to the fact that electro indifferent subjects do not even quite surely change their charge through aluminium salts. The charge is only diminished to zero. That has been found out in my laboratory for cellulose, agar, colloidon. Jacques Loeb pointed it out for collodion; only the quadrivalent thorium brought about a positive charge. As LOEB pointed out, these facts only change when such substances are covered with an amphoteric colloid as albumin, peptone, gelatin. Common amphoteric amino acids do not behave like that, because they are not adsorbed by the particles. In opposition to that, albumin

covers collodion with a wash proof layer at the superficies, and this condition being given, the superficies is no longer a collodion surface but a protein surface. Neglecting these peculiar cases the rule remains that collodion and such matters are always negative against aqueous phases, except in the presence of thorium.

Now we wish to explain how a double layer of ions may be formed at the surface of quite indifferent substances. A purely formal explanation is: indifferent substances adsorb negative ions better than positive ones. I need not emphasize once more that adsorption has only a formal meaning and does not involve the supposition that the adsorbent actively participates in adsorption: The real cause of this phenomenon is somewhat problematic. I recommended the following working-hypothesis. Supposed hydroxyl ions are more capillary active than hydrogen ions, any surface of water must be negatively charged against the interior of the water, provided this surface touches a body with no forces directed against the water which might disturb the distribution spontaneously aimed at. LOEB pointed out a similar idea: as we have been taught by LANGMUIR that molecules are often arranged in a definite direction when situated within the superficial layer of a liquid, one might suppose the superficial molecules of water to be directed with the hydroxyl groups outward and the hydrogen groups inward. For proving this hypothesis one should try to somehow confirm it experimentally or derive it somehow theoretically out of the polar nature of water molecules. Lately LOEB has investigated the influence on this potential, of the electrolytes added. He used such indifferent substances as collodion, graphite, gold, and estimated the potential from cataphoresis experiments. The negative potential was manifest even in pure water, but to a very small degree, scarcely surpassing about 7 millivolts. It increases by addition of any electrolyte and passes a maximum with increasing concentration, this maximum scarcely ever surpassing 60 millivolts. When the anions of the electrolyte are varied, the negativifying effect increases with the degree of valency; when the cations are varied, the negativifying effect decreases with the valency. The concentration increasing, the potential finally becomes equal to zero. The trivalent lanthanum in high concentrations may cause a doubtfully positive charge and only the quadrivalent thorium, even in lowest concentration effects an energetic positive charge. This peculiarity of thorium ions has been likewise pointed out in the charge of air bubbles against aqueous solutions by McTaggart. From my former investigations I derived a rule, that changing of the sign of charge by polyvalent ions might be characteristic of acidoids. We have to modify this rule slightly and we may summarize it in the following manner:

Particles capable of a dissociation tendency of an amphoteric character can change the sign of the charge according to the hydrogen ion

concentration; at a certain hydrogen ion concentration there is an isoelectric point. The sign of their charge is also changed by polyvalent ions, or in other words: that hydrogen ion concentration just causing the charge to be turned, is diminished in the presence of other ions, and especially of polyvalent cations.

2. Particles which may be considered as acids according to their chemical constitution do not change the sign of their charge by hydrogen ions in however high concentration: yet they do so by trivalent cations. (It seems to me not certain that bivalent ions are able to change the sign.)

3. Particles of quite indifferent nature such as air bubbles, collodion, cellulose, certainly do not change the sign of the negative charge by hydrogen ions and almost certainly not by trivalent cations, but only by quadrivalent cations.

Formerly we imagined that in a watery solution there is a statistic average, a homogeneous distribution of all kinds of ions, but the formation of electrical double layers at the boundary surface of an electrolyte solution and of chemically quite indifferent substances and even gases, compels us to admit, as a rule, a different capillary activity for the anions and the cations of an electrolyte, which brings about an electrical charge of the superficies of the solution without any exterior forces being active.

I wish I had the opportunity of speaking some more words about the adsorption and the potential difference in the case of charcoal as an adsorbent, which cannot be so easily fitted into the system given just now, but my time being limited, I shall rather proceed to a chapter of a more general interest, that is: The connection between the discharge of a colloid and the flocculation as a consequence of the discharge, on the one side, and the adsorption of that electrolyte, which causes the discharge and the flocculation. For we know that as a rule any electrolyte, which is added to a colloidal solution, diminishes the electrical charge of the particles and finally brings about coagulation.

FLOCCULATION

Why do electrolytes added to a colloidal solution lower the electrical charge of the colloidal micellae? Now experience has taught, that very often the added electrolyte is *adsorbed* by the colloidal particles, and so secondly we might suppose that the *adsorption of an electrolyte has some connection with its discharging power.* So let us begin the discussion of this most important question by discussing the connection between flocculation and adsorption. Again, we might elucidate the matter by an example according to an investigation about mastic sol, which I made many years ago. When you flocculate a mastic sol by an electrolyte, this electrolyte is always found to be partially adsorbed

by the flocculated particles when chemically analyzing them, except in two cases. These exceptions are: firstly: flocculation by acids; secondly: flocculation by common neutral salts of alkali metals. This second case, however, may be considered as doubtful for the following reason. Trivalent cations flocculate in a very low concentration, and adsorption occurs at a considerable rate, easiest accessible to chemical analysis. Bivalent cations bring about flocculation only in higher concentration; the absolute amount of the adsorbed cations may be the same as in trivalent cations, but the relative amount being much lower, adsorption can not be so easily proved by chemical analysis. But after all, we are just able to prove the adsorption. In univalent cations the concentration sufficient for flocculation is still much higher. Though these might be adsorbed at the same absolute rate as trivalent cations, this amount is no longer accessible to chemical analysis. Therefore, the fact that in univalent cations adsorption is not evident, is likely to be only the consequence of a technical insufficiency. In opposition to that, in the case of flocculation by acids, such an interpretation would not satisfy at all, and I wish to emphasize this fact on account of its great theoretical importance. A mastic solution is flocculated by a solution of hydrochloric acid extremely dilute, about 10^{-3} to 10^{-4} normality; the conditions of chemically analyzing the deficiency of the adsorbed acid are most favorable. However, acids cause flocculation without being adsorbed in the least. The most likely interpretation of this fact is an idea suggested just before. The cause is, that the hydrogen ions of the solution suppress the tendency of mastic to emit hydrogen ions and thus discharge the electrical double layer. The single micella of a mastic sol may be considered as an electrical spherical condensor. The interior couch is the negative superficial layer of colloidal anions, the exterior one is the couch of hydrogen ions. Though the latter does not represent a real surface in the mathematical meaning, we may, as for electrical effects, imagine it substituted by a real surface with a definite electrical density and a definite distance from the other layer. The higher the hydrogen ion concentration of the aqueous solution is, the smaller is the distance of the surfaces of the double layer from each other, and the smaller is the potential difference of the surfaces of the double layer. This potential difference being diminished to the critical value, flocculation occurs. ***In the flocculating effect of acids, the effect of diminishing the potential may be immediately understood without any adsorption interfering.*** However in all the other cases there is an evident connection of adsorption and discharge. As for the kind of adsorption, as a rule we are compelled to admit that kind of adsorption which I called adsorption by exchange or substitution or mutual adsorption. For instance, when a negative sol is suspended in a solution containing only univalent ions in low concentrations, is flocculated by adding a small amount of a bivalent ion as calcium, this calcium is

found partially adsorbed by the precipitate, and in this case we are allowed to admit that the bivalent cation has substituted some univalent ion which originally had formed the exterior surface of the double layer. Adsorption then means a substitution of univalent ions of the double layer by bivalent ions. Thus our question is reduced to the following question: Why is the potential of the double layer smaller when bivalent ions partake in forming the double layer, than when they do not. The difficulty of this case is that the exterior layer consists no longer of one single kind of ion but of a mixture of ions. For when we add increasing amounts of calcium chloride gradually to the sol, a *gradual* substitution by calcium ions, of the original hydrogen ions will occur and not before a certain excess of calcium is reached, the substitution will be practically complete. There is no reason to admit the substitution to be complete in the very concentration of calcium chloride just sufficient to bring about a flocculation. At first, a specific effect of calcium ions, different from that one of hydrogen ions, might seem to exist; however such an assertion is not available. For when, instead of calcium ions we add hydrochloric acid, flocculation occurs still easier; we need less hydrochloric acid than calcium chloride for a complete flocculation. Thus, the very substitution by itself is no sufficient cause for diminishing the potential; only it is not possible to substitute the hydrogen ions of the double layer by calcium ions by any other way but only by adding a relatively large amount of calcium chloride. The equilibrium of substitution, or in other words, the equilibrium of adsorption, demands a relatively high concentration of calcium ions within the solution, if a measurable amount of calcium ions be fixed in the double layer. In this case, we might say, the superficies of the micella is no longer the free colloidal acid, but partially its calcium salt. This calcium salt is coagulated through the calcium ions of the aqueous solution for the same cause, as the colloidal acid itself is coagulated by a sufficient amount of hydrogen ions within the solution, that is: by suppression of the tendency of dissociation.

When the exterior layer only consists of hydrogen ions, the potential is evidently a function firstly of the electrical surface density, that means the density of the hydrogen ions within the exterior layer of the double layer, and secondly of the concentration of the hydrogen ions within the aqueous solution. We are allowed to assert that, without being able to specialize the mathematical form of this function. Now, when the double layer consists partly of calcium ions and partly of hydrogen ions, the potential difference could be calculated either as a function of surface density and concentration of hydrogen ions *or* as a function of surface density and concentration of calcium ions. Either calculation must give the same value for the potential, provided adsorption equilibrium is established. So let us regard only the

hydrogen ions. As soon as some hydrogen ions of the double layer have been substituted by calcium ions, the surface density of the hydrogen ions in the double layer is diminished, while the concentration of the hydrogen ions within the solution remains practically constant. From that a decrease of the potential results, and that is the intrinsic cause of the fact known by experience, that adsorption of calcium ions is connected with a decrease of the potential.

From that it results, that the efficiency in decreasing the potential of any ion is the greater the more this ion is able to substitute the hydrogen ions of the double layer, which at the same time form the superficies of the aqueous solution against the solid particles, with other words, the greater the adsorbability of the ion. So the question about the discharging faculty of ions is reduced to the question about the adsorbability of ions.

In a colloid endowed with dissociation tendency, we may recognize one special kind of ions, that is that kind of ions, which the colloid has the tendency to send into the solution when suspended in pure water or at least in water containing *not* or *weakly* adsorbable ions. Suppose the colloid is an acidoid, this special ion will be the hydrogen ion; is the colloid an ampholytoid as albumin, this special ion will be either the hydrogen ion or the hydroxyl ion, according to the acidity or alkalinity of the solution. In other cases it seems that also other ions are necessary for the stability. So colloidal ferric hydroxide always contains chlorine ions, which are likely necessary to form the colloid besides the hydroxyl ions of the free hydroxide. We may call these special ions the original ions of the colloids. If the colloid is an acidoid the original ion is always the hydrogen ion, and this ion can only be substituted by other positive ions. Negative ions of the watery solution must be indifferent and without any remarkable efficiency, however they might be adsorbable by itself. You recognize the HARDY rule: only ions of opposite charge to the colloid have a coagulating effect of the colloid. Generally the adsorbability of ions increases with the valency in a high degree. You recognize the SCHULTZE rule: the efficiency of ions upon colloid increases with their valency.

Hitherto we started all theory of the electrifying effect of ions from the standpoint of adsorption. Once more I emphasize that this conception has a purely formal meaning, it was only a fulcrum for a systematical treatise. So we should not be surprised that the electrifying effect of ions may be considered from a quite different point of view, also, without contradiction. Now indeed in the last time a different theory has been developed, reducing the electrifying effects of ions at the surface of colloidal particles to the so called DONNAN equilibrium. I will discuss the problem how to reconcile this theory with my former ideas.

The following consideration seems to me to be the solution of this problem. Producing a potential difference by a DONNAN effect is nearly the same as producing a potential difference by dissociation tendency. The common principle is the fact, that a diffusible kind of ion is prevented from a free diffusion by an indiffusible colloidal ion. In the theory of dissociation tendency, the *original* supposition is that a solid particle is endowed with this dissociation tendency. This condition is mostly not given in a pure form. The micellae of a colloidal solution do not only contain that kind of molecules, which embody the colloidal behavior, but also water and salts. In a pure crystal of a salt, the dissociation tendency of either ion may be considered to be a constant. In a real colloidal micella, containing water and salts, the dissociation tendency of the colloidal electrolyte may depend on the *concentration* within the micella, of this colloid. But generally this solution not being a dilute one, but often even a very concentrated one, parallelism of concentration and chemical activity does not hold. In the case that the colloidal particle contains so much water, that it may be considered as a dilute solution, you may use the conception of "concentration" of the dissolved molecules, in the usual meaning of the word. But these conditions being given, the mechanism of the dissociation tendency is ruled by the DONNAN law. So you see, the so called DONNAN formula may be applied for a limited case of dissociation tendency, namely for colloidal micellae abundant with water.

I am conscious that this system of the electrical double layers in colloidal solutions may be incomplete and partially, perhaps doubtful. But you might consider it, at least, as a first attempt and though, perhaps wrong, yet useful for further investigations.

In all these cases there was a common principle that the ions which determined the properties of the colloid particles were situated in the adsorption sphere of the particles, whether these ions were really adsorbed from the surroundings or they were emitted by the particle through dissociation tendency. In all these cases the electrical properties prevailed, that is the sign of charge and the number of valency while specific chemical properties did not become surely evident *as a rule*. We shall now proceed to the effect of the ions of the solution which is exerted on the colloid particles at a distance, the water being the intermediary medium. These effects may be called the hydrophilic effects. They become evident only if the colloid has a certain degree of hydration and it is not to be seen in strictly hydrophobic colloids. This effect may be interpreted as a competition for the water of the colloid particles and all ions dissolved. This effect is not restricted to those ions of the *opposite* sign of charge to the colloid particles, but the sign of the charge is of no importance. Furthermore, there is no fundamental difference among the different ions according to their valency, such as it is in electrostatic effects. These lyotropic effects of

ions are very various and cannot be exhausted in a few words. Only there is a general principle in this effect.

When one orders the kinds of ions in a series according to the degree of hydrophilic efficieney, almost always the same order of series of ions appears, which is the same in all cases of hydrophilic effects. These orders are the lyotropic series or Hofmeister series. Here the valency is not such an important quality, and even among ions of equal sign and valency there are great differences. For instance we see an evident graduation in the lyotropic effect within the series of univalent cations, the hydrophile decreasing from Li to Na to K to Rb to Cs; and the bivalent cations are as a rule of no generally stronger efficiency, but Ca Ba Sr may be interpolated within the series between their first member Li, and the last member Cs.

Concerning the theory of the water attracting force of the different ions, 10 or 20 years the idea prevailed that each ion formed a real combination with a definite number of water molecules just so as an ion of a crystal grate is combined with a definite number of molecules of water of crystallization. However, we should prefer the newer idea that in *solutions* the ions are not combined with a *definite* number of water molecules but they have an attraction power toward all water molecules surrounding them, even at a distance. This attraction is based on the polarizing influence on the water molecules, which have the properties of an electric dipole. Now colloid particles also, in so far as they are hydrophilic, have this polarizing influence toward the water dipoles, and from that the competition of the micellae and the ions of the solution for the attraction toward the water arises. Hence the ions of the solutions influence all those properties of a hydrophilic colloid, which depend on the hydration of the colloidal micellae. These qualities are: degree of dispersity, viscosity, swelling, jellification and others. As it is quite impossible to exhaust this problem in such a paper I should prefer to elucidate the matter through two examples, which seem to be especially instructive for general views.

The first example concerns the swelling of an agar jelly, which I asked Dr. Dokan to investigate in my laboratory. The behavior of agar is much more comprehensive than the one of gelatine, which has been mostly investigated, because agar is always electro-negative and shows no isoelectric point as gelatine does, this isoelectric point being a turning point for many properties. It is determined by a definite pH and requires an accurate consideration of pH in each experiment, which is much less necessary in agar. The results of these experiments on swelling of agar jellies were as follows: The swelling is most in pure water. Any added electrolyte diminishes the swelling. We may discriminate two ranges of concentrations of the added electrolytes, the first from extremely diluted solution up to about 0.01 or even 0.1N solution, the other from 0.1N up to 1N concentration and above.

For the lower range of concentration the same rules hold which Van Kruyt and deJong pointed out for the *viscosity* of very dilute agar sols, viz: The anion is of no importance at all, whether it is monovalent or polyvalent. In cations, only the valence number shows a clear effect. All univalent ions have the same effect, the molar concentrations being the same, all bivalent ions have a stronger effect, etc. Only the hydrogen ion is exceptional, its efficiency is still higher than the one of trivalent ions. At this range of ion concentrations all effects may be interpreted as electrical effects of those ions which participate in forming the double layers. But in *higher* concentrations this law becomes more and more invalid. Here the anions begin displaying an own effect, and the effect of the cation no longer depends on the valence number. The cations may be ordered to a series according to their efficiency in which the valency scarcely plays a rôle and in which even ions of equal sign and valence number are very unlike in the effect, such as Li Na K Rb, or SO_4, Cl Br. Here the Hofmeister series of the ions, or the lyotropic series becomes manifest, and this may be interpreted by a competition of the colloid particles and the ions of the aqueous solutions for the water. This effect is brought about by those ions which are *not* combined or adsorbed by the colloid particles. This lyotropic effect cannot be demonstrated in the lower ranges of ion concentration, in the case of the swelling of agar, and there is the direct electrostatic effect alone. But the lyotropic effect becomes evident in higher concentrations. This lyotropic effect can be shown even in such cases where a direct electrostatic effect does not exist at all. Such a case was found in my laboratory for a colloidal material called "Konyaku," a Japanese food stuff. It can be obtained as a jelly from a plant, Amorphophallus Konyaku, and it is a polysacharide. The swelling of this material is not at all influenced by any electrolyte up to a concentration of 0.1N; except for sodium hydroxide, which depresses the swelling. It is probable that this colloid has no electrical charge at all except in the presence of many hydroxyl ions. A direct electrostatic effect of any ion is lacking. But in higher concentrations, about 1N, the effect of different electrolytes on the swelling is quite enormous and does not depend on the valence number or the sign of the charge, but follows the Hofmeister series of the ions. For instance, the strongly water attracting Li causes an enormous shrinking of the Konyaku jelly, while Na and K have a very small effect. Some ions even increase the swelling enormously in comparison with the swelling in pure water, viz: Ba or I′, which bring about a complete peptization of this jelly. Such an effect may only difficultly be explained by our representations on hydrophily. But certainly, it belongs to hydrophilic effects. This colloid is very fit to demonstrate purely lyophilic effects as there are no electrostatic effects at all, as a rule.

The general result of these considerations is the following. For the influence of electrolytes on colloids two kinds of influences may be clearly discriminated:

1. The electrostatic effect, which almost entirely depends on the valency of the ions and which is almost entirely exerted by the ions of a charge opposite to the charge of the colloid. This influence may be interpreted by admitting that the ions form or at least participate in forming the electrical double layer surrounding the particles.

2. The lyotropic effect of the ions which becomes the more manifest the more the colloid is a hydrophilic one. This influence does not depend on the sign of the charge, nor does it depend on the valency in a clearly remarkable manner, but on the water attracting power of the ions. This influence becomes evident only in relatively high concentrations of the electrolytes, while the electrostatic influence may occur even in smallest concentrations.

Of course, even the lyophilic effect is an electrostatic effect too, originally; it depends on the dipolar nature of the water molecules. But in the case of a direct electrostatic effect we have to do with the attraction by free electrical charges which are present in the form of free ions. In the cases of indirect effects we have to do with forces exerted by an ion upon a dipole, that is the water molecule.

Let us try if these general remarks may be useful in colloid chemistry.

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THE ELECTRO-VISCOUS EFFECT IN RUBBER SOLS

G. S. Whitby and R. S. Jane

Studies of the influence of small amounts of electrolytes on the viscosity of lyophile sols have, with few exceptions,[1] hitherto been confined to hydrophile sols, such as sols of agar-agar, starch, casein, and gelatine. As the present (preliminary) communication shows, however, sols of rubber in benzene are affected in their viscosity by the addition of minute quantities of both organic acids and organic bases to an extent that would perhaps hardly have been expected in view of the fact that the ionizing power of the medium is extremely low and that there is little difference in dielectric constant between the two phases of the system.

In considering the effect of very small additions to benzene sols of rubber, it would appear to be desirable to distinguish between four types of added substances,[2] namely:

(*a*) Diluents,
(*b*) Precipitants,
(*c*) Reactants,
(*d*) Electrolytes.

A reference to some experimental results will illustrate the effects produced by substances falling in these various classes.

For all the viscosity measurements given in this paper the temperature was 25°; the benzene used was dried over sodium wire; the rubber (pale crêpe) used was dried in a vacuum desiccator; an Ostwald viscosimeter was used; sols were, unless otherwise indicated, allowed to stand for 10-12 hours after additions had been made before their viscosity was measured; the results are expressed as relative viscosity referred to benzene (ratio of the times of flow). Since, as appears below, the colloidal condition of rubber sols is highly susceptible to the presence of minute quantities of acids and bases, possibly a few of the values obtained were affected by impurities in the materials used.

[1] *Cf.* Kruyt and Eggink, *Proc. Roy. Acad*, Rotterdam, 1923, 26, 43; Eggink, *Rec. trav. chim.*, 1923, 42, 317; various papers on cellulose sols; etc.

[2] Attention is here confined to the influence of chemical agencies on rubber sols. Several physical agencies are, of course, known to reduce the viscosity of rubber sols. Of such agencies reference may be made to ultra-violet radiation. On exposing a benzene rubber sol in a quartz tube for 20 minutes to a mercury arc at a distance of 10 cms., the authors found the time of flow of the sol to be reduced from 5 mins. to 2 mins. 38.2 secs. Exposure for 10 minutes to X rays produced no fall in viscosity. The manner in which ultra-violet rays acts remains to be explained.

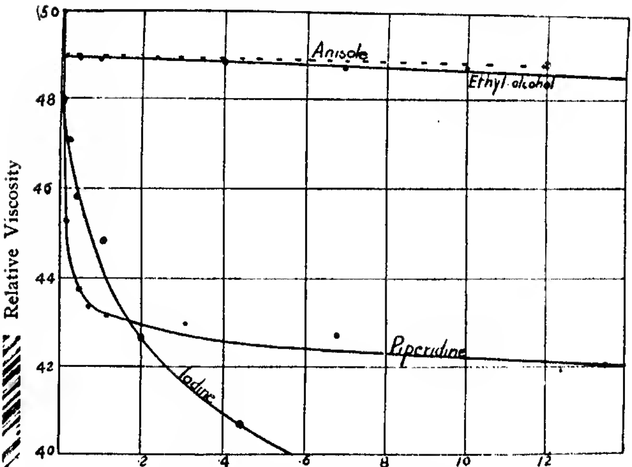

FIG. 1.—Milligrams per 10 c.cs. of rubber solution.
(Cold-extracted rubber used.)

In most cases of doubt, however, materials were subjected to special purification. On account of the susceptibility just mentioned, it is necessary to work in a laboratory free from acid and ammonia fumes, as the presence of such when sols are being poured from one vessel

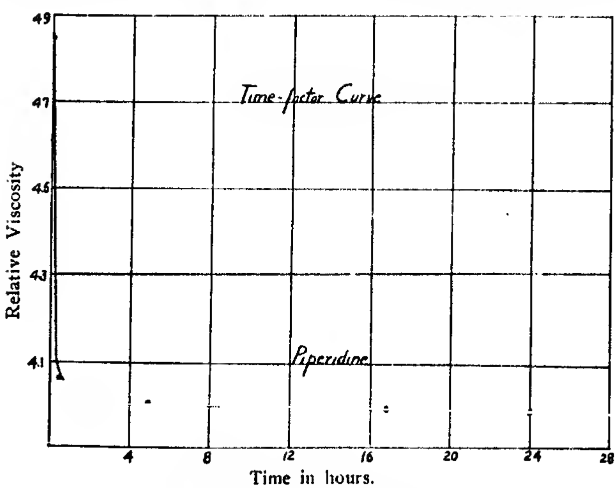

FIG. 2.—Amount added = 120 milligrams per 100 c.cs.
(Cold-extracted rubber used.)

to another or otherwise handled noticeably affects the viscosity values obtained.

Diluents. By diluents is meant non-electrolytes which, like benzene itself, are swelling agents and will not precipitate rubber from its solutions. Minute quantities of such substances, of the order of the quantities of electrolytes which affect the viscosity, alter the viscosity of benzene rubber sols only slightly. Ethers and mustard oils may perhaps be taken as typical of such substances. A curve in Fig. 1 shows the hardly appreciable effect of small amounts of anisole on the viscosity of a benzene rubber sol. Even when added in very much larger proportions than the proportions of electrolytes necessary to influence the viscosity (*vide infra*), the effect of such substances is only small, as the results given in the following Table show.

TABLE I

INFLUENCE OF ETHERS AND MUSTARD OILS ON THE VISCOSITY OF A BENZENE SOL CONTAINING 0.323 GMS. UNEXTRACTED RUBBER PER 100 C.CS.

Substance added[3]	Amount per 100 c.cs.	Relative viscosity
None		4.86
Phenetole	140 mgs.	4.89
Benzylethyl ether	106 "	4.77
Isoamyl ether	80 "	4.69
Anisole	126 "	4.56
Ethyl mustard oil	150 "	4.90
Allyl mustard oil	125 "	5.11

Precipitants. The number of liquids capable of precipitating rubber from its solutions is very limited. Ethyl alcohol is one of the best-known of such liquids. The curve for alcohol in Fig. 1 shows that when added in very small proportions alcohol has practically no effect on the viscosity.[3a]

The effect on the viscosity of rubber sols of various substances which may change the degree of solvation of the disperse phase is a matter that would appear to deserve further study. Ethers and mustard oils, to which reference has been made, are agents the swelling of rubber in which is of the same order as its swelling in benzene. Alcohol, on the other hand, is an agent which is capable of desolvating the disperse phase to such an extent that the rubber separates. It should be interesting to examine the influence of non-electrolytes the swelling

[3] None of the substances (Kahlbaum) was subjected to purification; and possibly the results for anisole and allyl mustard oil in particular are influenced by the presence of impurities. In later experiments, the anisole, after being shaken with water and redistilled, had practically no effect on the viscosity of a sol.

[3a] The immediate effect of adding alcohol is to reduce the viscosity appreciably, but, on allowing the sol to stand for several hours, the viscosity gradually rises and comes within 12 hours to constant values, such as the values shown on the curve. The initial fall is presumably due to local de-solvation produced at the point of entry of the alcohol; and on standing the portion of the disperse phase which has thus fallen in its degree of solvation swells again, and consequently the viscosity rises.

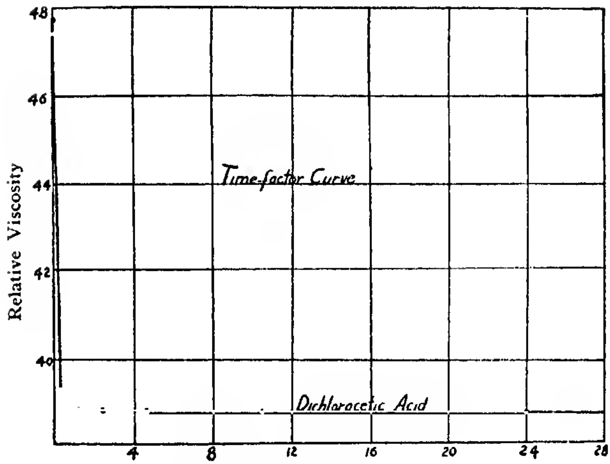

FIG. 3.—Amount added = 130 milligrams per 100 c.cs. (Cold-extracted rubber used.)

action of which is less than that of benzene, the ethers, or the mustard oils but greater than that of alcohol.

Reactants. Electrolytes. The effect on the viscosity of rubber sols of, on the one hand, substances which undergo chemical reaction with caoutchouc and on the other hand electrolytes (especially organic acids and organic bases) appears to be definitely distinguishable by

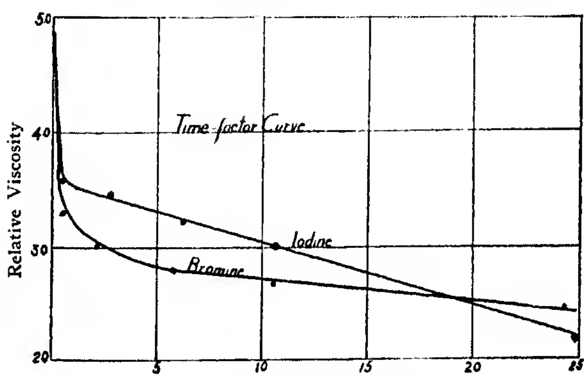

FIG. 4.—Amount added = 50 milligrams per 100 c.cs. (Cold-extracted rubber used.)

examination (*a*) the change of viscosity with time; (*b*) the change of viscosity with increasing amount of the added substance.

Organic acids and organic bases produce their effect on the viscosity practically at once, as is shown by the time curves for piperidine and for dichloracetic acid given in Figs. 2 and 3 respectively. The effect on the viscosity of substances which react chemically with caoutchouc is not thus immediate, but, instead of coming sharply to an end, continues over a long period of time, as the progressive fall in viscosity produced by iodine and by bromine (shown in Fig. 4) demonstrates. The curves representing the influence (over 10-12 hours) of increasing amounts of substances in the two classes, viz. reactants and electrolytes, are similarly contrasted. The curves for organic acids and organic bases flatten out, addition of these substances beyond a certain, small amount producing but little additional effect. This is shown by curves in Figs. 1, 6, and 7. The curves for reactants, on the other hand, do not flatten out in the same way. Whereas a given organic acid or organic base is capable of reducing the viscosity only to a certain extent, no matter how much is added, increasing amounts of iodine and bromine produce progressively increasing reductions in viscosity. Figure 1 enables a comparison to be made of an electrolyte, piperidine, and a reactant, iodine.

The proportion of a reactant, such as iodine, which is capable of reducing the viscosity is surprisingly small and would indicate that the effect is not ascribable merely or directly to the formation of addition compounds, such as $(C_5H_8I_2)_n$. For example, as may be seen from Fig. 1, 2.18 mgs. of iorine per 100 *c.cs.* of a sol containing 0.496 grams of rubber reduced the viscosity from 4.80 to 4.27; and this amount of iodine corresponds to only 0.12 per cent of the theoretical amount required to convert the caoutchouc present into $(C_5H_8I_2)_n$. In some further experiments an amount of iodine corresponding to only 3.24 per cent of the amount required to saturate the caoutchouc present reduced the viscosity from 4.85 to 2.94. It may further be remarked that with sols to which iodine or bromine have been added it is difficult to get concordant results in repeat measurements. It was observed, for example, that the time of flow of a sol initially about 5 minutes might change as much as 10 seconds when the same sol was run through the viscosimeter a second time. With sols to which bases or acids had been added no such difficulty was encountered.

Another reagent, presumably a reactant, which brings about a great fall in viscosity is tetranitromethane. 1 drop of this added to 10 c.cs. of a sol with an initial relative viscosity of 4.85 reduced the viscosity (measured after 24 hours' standing) to 1.70.[3b]

The remainder of this paper will be devoted to the fourth class of chemical reagents affecting the viscosity of rubber sols, *viz.* electrolytes.

[3b] Another reactant which was observed to reduce the viscosity is benzoyl peroxide.

Acids. That the addition of benzoic and of acetic acid to benzene rubber sols reduces the viscosity has been shown by Kruyt and Eggink.[4] The present authors have examined the effect of small quantities of a number of organic acids the dissociation constants of which in aqueous solution are on record. A comparison of the effects of acetic acid, monochloracetic acid, dichloracetic acid, and trichloracetic acid is shown in Fig. 5. The order of effectiveness of these acids in reducing the viscosity of a benzene rubber sol is the same as that of the dissociation constants (shown below) determined in water.

	Dissociation constant in water
Acetic acid	1.8×10^{-5}
Chloracetic acid	1.55×10^{-3}
Dichloracetic acid	5×10^{-2}
Trichloracetic acid	3×10^{-1}

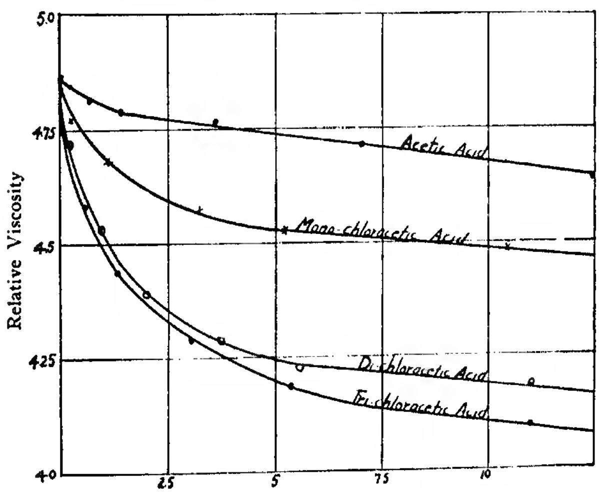

FIG. 5.—Milligrams per 100 c.cs. of rubber solution. (Cold-extracted rubber used.)

Some data for benzoic acid, o-nitrophenol, picric acid, and certain fatty acids, *viz.* caprylic, stearic, heveïc, and oleic acids, are given later.

The measurements with acetic acid and its chlorine derivatives shown in Fig. 5 were made on a sol prepared from a sample of rubber from which the greater part of the fatty acids naturally present in rubber[5] had been removed by extraction for a week at room temperature with a 1 : 1 mixture of acetone and ether, the extractant being renewed every

[4] *Loc. cit.*
[5] Whitby, *India-Rubber J.*, 1924, **68**, 617, 735.

24 hours or so. It appears that the magnitude of the reduction of viscosity produced by organic acids and bases is influenced to some extent by extraction of the rubber with acetone or with a mixture of acetone and ether. In sols prepared from rubber cold-extracted in the way described above the reduction is smaller than in sols prepared from unextracted rubber; and in sols prepared from rubber which has been subjected to extraction with hot acetone the effect is still smaller, as the results given in the following Table show. The amount of reagent added was in all cases large enough to produce substantially the maximal effect of the reagent in question.

TABLE II

(Figures in brackets show, in mgs. per 100 c.cs., the amount of reagent added.)

Reagent added	Dissociation const. in water	Relative Viscosities			
		Sol No. 1 From unextracted rubber. Concn.: 0.328 g. per 100 c.cs.	*Sol No. 2* From cold-extracted rubber (No. 1). Concn.: 0.496 g. per 100 c.cs.	*Sol No. 3* From cold-extracted rubber (No. 2). Concn.: 0.286 g. per 100 c.cs.	*Sol No. 4* From hot-extracted rubber. Concn.: 0.568 g. per 100 c.cs.
None	---	4.86	4.86	4.37	4.39
Dichloracetic acid	5×10^{-2}	(150) 3.42		(150) 3.26	(150) 3.68
Benzoic acid	6.6×10^{-5}	(100) 4.05	(100) 4.26	(100) 3.80	(100) 4.05
Acetic acid	1.8×10^{-6}	(100) 4.00	(100) 4.48		
o-Nitrophenol	6.8×10^{-8}	(100) 4.26	(100) 4.86	(100) 4.38	(100) 4.47
Picric acid	1.6×10^{-1}	(100) 4.30			

In view of the fact that fatty acids (heveïc, oleic, and linolic) occur naturally in rubber,[5] it is of interest to enquire whether such acids appreciably affect the viscosity of benzene rubber sols. The following Table shows some results obtained with a number of fatty acids, the rubber used being unextracted.

TABLE III

INFLUENCE OF SOME FATTY ACIDS ON THE VISCOSITY OF BENZENE SOLS OF UNEXTRACTED RUBBER

Caprylic acid		*Stearic acid*		*Heveïc acid*		*Oleic acid*	
Amount (mgs./100 c.cs.)	Relative viscosity	Amount (mgs./100 c.cs.)	Relative viscosity	Amount (mgs./100 c.cs.)	Relative viscosity	Amount (mgs./100 c.cs.)	Relative viscosity
0	4.86	0	4.85	0	6.24	0	6.325
150	4.14	2	4.63	2	5.93	36	6.07
		8	4.35	7	5.83	59	5.88
				30	5.72		
				50	5.67		

The total amount of fatty acid naturally present in rubber is of the order of 5 mgs. per 100 c.cs. of sols such as those used. The above

data make it clear that additions to the naturally-present acid reduce the viscosity. It has frequently been proposed to use measurements of the viscosity of benzene sols of different samples of raw rubber as a means of estimating the vulcanizing quality of the samples. The influence of the natural-acid-content of rubber (which varies in different samples) on the viscosity has, however, been overlooked. This aspect of the matter has been discussed more fully elsewhere.[6]

Bases. It has been found that not only organic acids but also organic bases reduce the viscosity of benzene rubber sols. As in the case of strong organic acids, the effective amounts of strong bases are extremely small, and increase beyond a certain point of the amount of reagent

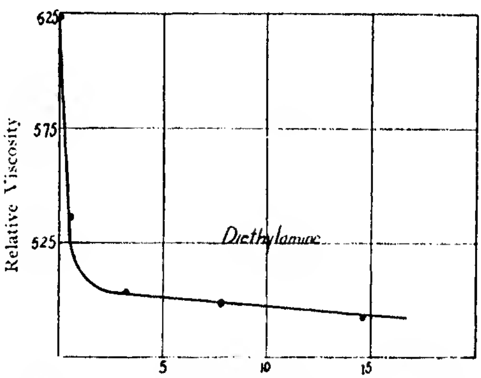

FIG. 6.—Milligrams per 100 c.cs. of solution of unextracted rubber.

added has little additional effect. This is seen from the data, given in Figs. 6 and 7, for diethylamine. Thus, for example, 0.004 gms. of diethylamine added to a benzene rubber sol of initial viscosity 8.85 reduced the viscosity to 6.37. In Fig. 1 are shown results for piperidine, using a rubber sol containing 0.496 gms. of rubber per 100 c.cs. It may be seen, for example, that 0.0004 gms. of piperidine per 100 c.cs. sol reduces the viscosity from 5.000 to 4.375.

A comparison was made of the effect on the viscosity of a number of bases of different strengths (as judged by their dissociation constants determined in water). The amounts of the various substances added were comparatively large: that is to say: amounts falling, at least in the case of the strong bases, well on the flat portion of the curve connecting viscosity and amount of added reagent. The results are shown in the following Table. The rubber sol used was Sol. No. 1 mentioned in Table II.

[6] Whitby, *loc. cit.* *Cf.* De Vries, *Ibid.*, May 24, 1924.

TABLE IV

INFLUENCE OF BASES ON THE VISCOSITY OF A BENZENE SOL OF UNEXTRACTED RUBBER

Base	Dissociation const.	Amount (mgs. per 100 c.cs.)	Relative viscosity
None	—	—	4.86
Piperidine	1.6×10^{-3} (25°)	130	3.73
Diethylamine	1.26×10^{-3} (")	116	3.84
Dipropylamine	1.02×10^{-3} (")	96	3.77
Di-isobutylamine	4.8×10^{-4} (")	114	3.91
Isobutylamine	3.1×10^{-4} (")	114	3.88
Benzylamine	2.4×10^{-5} (")	186	4.04
Dibenzylamine	—	171	4.04
Quinine	2.2×10^{-7}	100	4.47
Methylaniline	7.4×10^{-9}	171	4.77
Pyridine	2.4×10^{-10} (15°)	168	4.45
Aniline	1.7×10^{-10} (")	188	4.88

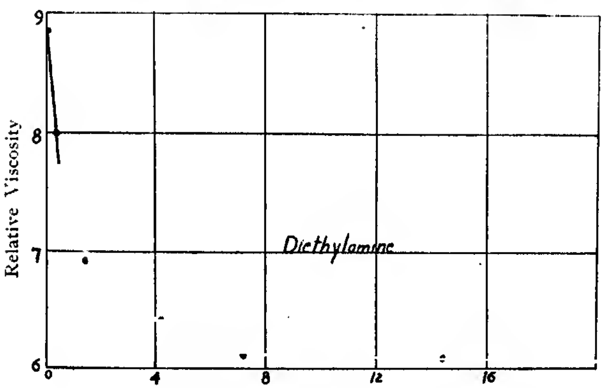

FIG. 7.—Milligrams per 100 c.cs. of solution of unextracted rubber.

With the exception of pyridine (the result for which was confirmed in two re-determinations), the order in which the thirteen bases in the above Table fall as regards their influence on the viscosity of the rubber sol closely follows the order in which they fall when ranged according to their dissociation constants measured in aqueous solution.

DISCUSSION

Influence of acids and bases on swelling. In considering the question of the manner in which organic acids and bases affect the viscosity of rubber sols, the point may first be raised as to whether it is through an influence on the degree of solvation of the disperse phase. Actual experiments on the effect of the acids in question on the swelling of

rubber in benzene showed the acids to have little or no effect on swelling when present in proportions at least as great as the proportions used in the viscosity experiments. The swelling measurements were made on samples of vulcanized rubber (prepared from a 90: 10 rubber-sulfur mixture). The swelling of raw rubber is in general parallel to that of vulcanized rubber but greater. The following Table shows results obtained.

TABLE V

Effect of Acids on the Swelling of Vulcanized Rubber in Benzene

Substance added	Amount per 100 c.cs.	Swelling (gms. liquid imbibed per gm. rubber)
None	--	3.31
Acetic acid	100 mgs.	3.30
Dichloracetic acid	140 "	3.49
Benzoic acid	100 "	3.48
Stearic acid	100 "	3.57

The influence of small amounts of strong organic bases on the swelling of vulcanized rubber (90: 10 rubber-sulfur vulcanizate) was next examined. The following results show that the very small proportions of piperidine which are effective in bringing about substantially the maximal reduction of viscosity which piperidine is capable of producing (cf. Fig. 1) have no appreciable effect on swelling. They reveal, however, the fact that in somewhat larger amounts strong organic bases have a very striking effect on the swelling. The pieces of vulcanized rubber used weighed 0.087-0.089 gm.; they were immersed in 5 c.cs. of benzene; and the swelling was measured after 24 hours' immersion.

TABLE VI

Influence of Bases on the Swelling of Vulcanized Rubber

Base	Amount per 100 c.cs. benzene	Swelling (gms. liquid imbibed per gm. rubber)
Piperidine	0	3.98
	1.3 mgs.	3.98
	2.7 "	4.02
	6.5 "	3.98
	13.5 "	4.11
	27 "	4.17
	70 "	4.19
	140 "	4.51
	220 "	4.86
	940 "	6.60
	1880 "	7.82
None	—	3.50
Piperidine	130 mgs.	5.00
Diethylamine	116 "	5.61
Dipropylamine	130 "	4.01

Since small amounts of organic acids, such as the amounts which are effective in reducing the viscosity of benzene rubber sols, and since minute amounts of organic bases, such as are similarly effective, have little or no effect on the swelling of rubber (Tables V and VI); since such influence as organic bases have on swelling is in the direction of increasing rather than in the direction of diminishing it,[7] it would appear that an explanation of the action of such substances on the viscosity of rubber sols must be sought elsewhere than in the direction of their influence on the degree of solvation.

It would seem, then, that the effect of organic acids and bases on rubber sols is to be regarded as due to ions, *i.e.* as an electro-viscous effect, arising from the neutralization of charges (presumably negative [8]) on the disperse phase by absorbed ions. This view would be in accord with the apparent fact that the order of activity of individual acids and of individual bases is, broadly speaking, the order of their dissociation constants (determined in water). There are, however, several noteworthy aspects to the matter.

The magnitude of the effects observable is unexpectedly large, in view of the low dielectric constants of both rubber and benzene. For, in the first place, the difference between the dielectric constants of rubber (2.12) and of benzene (2.29) is so small that the charge present on the disperse phase might be expected to be very small; and, in the second place, benzene is such a poor ionizing medium that in it the degree of dissociation of even strong acids and bases must be small.

Another noteworthy feature is that both organic acids and organic bases reduce the viscosity of benzene rubber sols. If it is assumed that acids exercise their effect by virtue of hydrogen ions to which they give rise, then it would appear to be necessary to suppose that bases also act by virtue of the cations to which they give rise and which are presumably absorbed in preference to their anions. In any case there arises the problem—What are the ions to which amines give rise in benzene?

It is unlikely that in the dried benzene used there would be sufficient water to enable the bases to ionize to substituted-ammonium cations and hydroxy anions (and, even if such ions did arise, it would still seem to be necessary to assume that the organic cation is adsorbed in prefer-

[7] The action of organic bases in increasing swelling will be examined more fully later. It may be pointed out that both the strong organic acid, dichloracetic acid, and the strong organic base, piperidine, are excellent swelling agents for rubber (both produce much more swelling than benzene). Yet small amounts (say, 1%) of the former added to benzene has little or no effect on the swelling, while small amounts of the latter greatly increases the swelling (Tables V and VI).

It may be mentioned that the viscosity of a sol of rubber in piperidine is higher than that in benzene. The following figures were obtained for sols of the same rubber-content:

	Time of Flow/Time of Flow of Benzene	*Time of Flow/Time of Flow of Piperidine.*
Benzene sol	4.86	2.14
Piperidine sol	7.67	3.47

[8] Cataphoresis experiments with benzene rubber sols gave no results when a potential difference of 110 volts was used.

ence to hydroxy ion). It is, too, unlikely that the effects are explicable by the presence of ions, of the types $R_2NH_2\cdot$ and A^-, derived from salts produced by reaction of the amine (say, R_2NH) with acid (say, HA) naturally present in the rubber; for in the cold-extracted rubber sample used the amount of natural acid left was too small to make an explanation on such lines probable. Thus, even in the case of piperidine, which was the most active of the bases examined, the amount of acid left in the cold-extracted rubber was such as to be equivalent to the presence of only about 0.16 mgs. of piperidine per 100 c.cs.—an amount insufficient to produce the full fall in viscosity observed. (The first

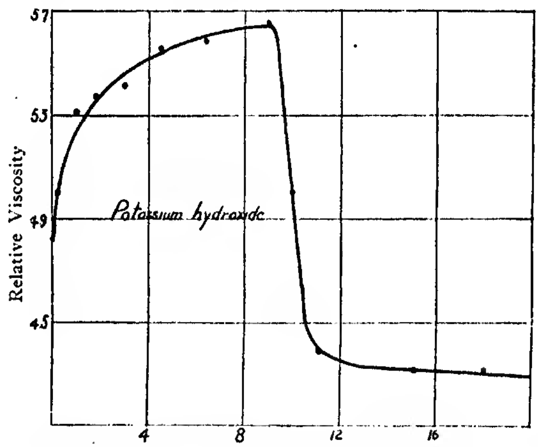

FIG. 8.—Milligrams per 100 c.cs. of solution of cold-extracted rubber. (0.4961 g. rubber per 100 c.cs.)

point on the piperidine curve in Fig. 1 corresponds to 0.2 mgs. of piperidine.)

It is of interest to note that the effect of electrolytes on the viscosity of benzene rubber sols finds a general parallel in the effect of electrolytes on electro-endosmosis in water, for in the latter phenomenon both acids and organic bases have been found to act in the same direction, viz. to cause a reduction.[9] The parallel extends to the action of caustic alkalis. As recorded in the following paragraph, increasing amounts of potassium hydroxide added to benzene rubber sols produce at first a rise and then later a sudden fall in viscosity. In a parallel way, caustic alkalis added in increasing amounts at first enhance electro-endosmosis and later reduce it.[9] Picric acid, which (*vide* Table II) has an effect on the viscosity of rubber sols much smaller than its dissociation constant would

[9] *Cf.* Elissafoff, *Z. phys. chem.*, 1912, 79, 385.

indicate, was found[10] to produce also an anomalously small effect on electro-endosmosis.

Potassium hydroxide. The effect of potassium hydroxide (introduced in alcoholic solution, one drop of solutions of suitable strengths being added to 10 c.cs. of benzene rubber sol), shown in Fig. 8, is markedly different from the effect of organic bases. In very small amounts it produces a rise in viscosity; as the amount is increased, the viscosity suddenly falls to below its initial value. Similar results were obtained in two repeat sets of measurements. The explanation of this course of affairs is not clear. The initial rise may be due to an increase in the negative charge on the disperse phase due to adsorption of hydroxy ions (which, it would have to be assumed, are more readily adsorbed by the rubber-benzene micelle than are potassium ions); or the course of the phenomena may be dependent upon the circumstance that the potassium hydroxide does not actually dissolve and ionize in benzene but is present only in colloidal solution; or still other factors may be concerned.

Kruyt and Eggink found ammonia to raise the viscosity of rubber sols. In a single experiment the present authors confirmed this observation.

The influence of salts on the viscosity of rubber sols will form the subject of a further communication. It may, however, be said that in preliminary experiments the curves connecting viscosity with concentration of added reagent show with a number of salts examined (viz. aluminium oleate, thorium oleate, and ferric chloride) a course similar to that found with potassium hydroxide.

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[10] Elissafoff, *loc. cit.*, 405.

DETERMINATION OF DISTRIBUTION OF PARTICLE SIZE

By W. J. Kelly

In studying the properties of pigments and fine powders it is often necessary to know, not only the average size of the individual particles, but also the percentage of particles of various sizes present in the powder. Several microscopic methods, such as the count method, have been devised, but these give only the average size and do not permit the determination of the distribution of sizes. It is usually more convenient to study the pigments and powders in liquid suspensions, and hence the most natural way of determining the size of particle would be to measure the rate of settling in a liquid and by the use of Stokes' law, so far as it is applicable calculate the diameter.

Previous Methods

Oden[1] has worked out a very ingenious method for weighing the sediment forming at the bottom of a tube, and from the rate at which it formed he was able to calculate the diameter and also the distribution of the particles of different diameters. Svedberg and Rinde[2] improved this method to the extent of adding an automatic weight recorder which drew a practically continuous curve. Figures are given by Svedberg and Rinde showing the distribution of particle size for gold and mercury hydrosols. The great advantage of this method is that very small amounts of material can be used. However, if large amounts of material are available it is usually more convenient to operate with slightly more concentrated suspensions provided they are still dilute enough so that no flocculation takes place.

A rough method was devised by Wo. Ostwald and von Hahn[3] for determining the rate of settling, but in this method the use of very concentrated suspensions, as high as 20 per cent solids, is necessary. The method depends on the fact that a suspension is specifically heavier than the medium, and hence, if the suspension is placed in one arm of a U tube and the suspending medium in the other, the latter will stand at a higher level. As the solid settles out the suspension becomes specifically lighter and the level difference in the two arms of the tube decreases. From the rate at which the level difference decreases a rough

[1] *Proc. Roy. Soc., Edinburgh*, 36, 219 (1916).
[2] *J. Am. Chem. Soc.*, 45, 943 (1923).
[3] *Kolloid-Z.*, 32, 60 (1923).

idea of the rate of settling can be obtained. Von Hahn used a tube such as is shown in Fig. 1. The suspension is placed in the left-hand tube and the medium in the right. A scale is mounted at the back so that the menisci can be read. The side tube is about 130 cm. long and the whole apparatus about 150 to 160 cm. In order to get a readable level differ-

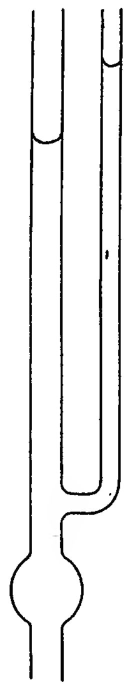


FIG. 1.—Sedimentation tube (von Hahn).

ence very concentrated suspensions were used. In these suspensions there was considerable flocculation and as a result the method measured more the rate of flocculation plus settling than that of settling alone.

PRESENT METHOD

The method described in this paper is a modification of von Hahn's, which permits the use of 0.5 to 1 per cent suspensions and by which the actual weight of the material settling past the entrance of the side tube can be calculated.

In a tube of the type shown in Fig. 2 the difference in level, a, in the two arms is given by the equation

$$a = \frac{D}{d} h - h \tag{1}$$

where h is the height of the suspension, D its density, and d the density of the medium or the liquid in the side tube. In case of short tubes and dilute suspensions a is very small. If the side tube is bent over,

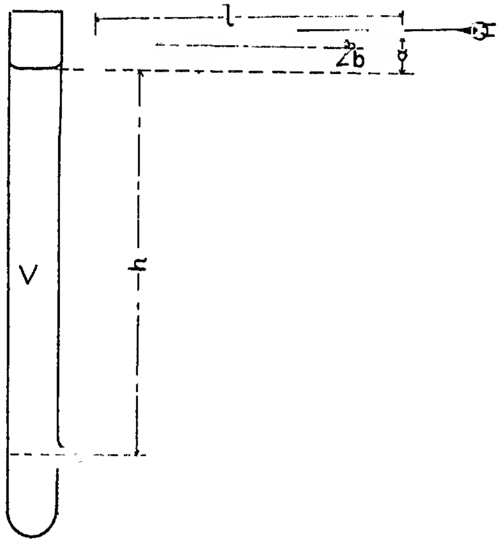


FIG. 2.

the apparent value of a can be increased considerably and measured in terms of the length of the liquid column in the horizontal part of the side tube. If this length is l, then

$$a = l \sin b \tag{2}$$

where b is the angle which the side tube makes with the horizontal.

In order to calculate the weight of material which settles past the side tube, the density of the suspension, D, has to be known in terms of the medium and the specific gravity of the suspended material. Thus

$$D = \frac{Vd - vd + w}{V} \tag{3}$$

where V is the volume of suspension in the large tube above the side

tube, v the volume of the pigment and hence also that of the medium displaced, and w is the weight of the solid phase.

If S is the specific gravity of the pigment, then

$$S = \frac{w}{v} \text{ or } v = \frac{w}{S} \tag{4}$$

Substituting (4) in (3)

$$D = \frac{Vd - \frac{wd}{S} + w}{V} = \frac{SVd - wd + wS}{VS} \tag{5}$$

Substituting (5) and (2) in (1)

$$l \sin b = \frac{h}{d} \, \frac{SVd - wd + wS}{VS} - h$$

which on simplification gives

$$w = \frac{dVSl \sin b}{h(S-d)} \tag{6}$$

In this equation w and l are the only variables for any given experiment, and as soon as the values of the constants have been determined the equation may be written

$$w = K.l \tag{7}$$

in which form it is easily used. The total weight of solid phase in the suspension being known, it is a simple matter to calculate the percentage which settles out in a given time.

In using this method the actual length of the side tube is immaterial, provided it is long enough to take care of the recession due to the settling. The zero point is taken at the upper end of the tube and the difference between this point and the position of the meniscus at any given time is taken as l. In this way the effect of capillarity is eliminated.

Precision Discussion

In Equation 6 all values with the exception of l and $\sin b$ can be determined so accurately that no error in w arises from them. For the values of b obtaining in the determination an error of 1 per cent in measuring the angle which is about 1 degree 30 minutes will introduce an error of approximately the same magnitude in the percentage of material settling out. The position of the meniscus in the capillary can be read to ± 0.01 cm., so that at the start a fairly large error can

be introduced from this value. However, as the recession in the capillary proceeds this error grows less, and at 2 cm. has been reduced to 1 per cent. In the example given 18 per cent of the material has settled out when the recession attains 2 cm., so that at this point the weight of material is known within 1 per cent.

For the calculation of the size of the particle it is assumed that Stokes' law is valid, and hence any error in the calculation will be only that inherent in the law itself.

Operation

The tube and capillary are first cleaned with potassium bichromate-sulfuric acid mixture and then rinsed thoroughly with distilled water.

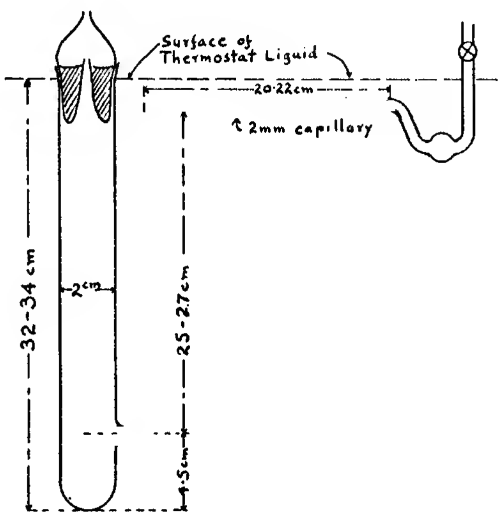


Fig. 4.

The whole tube is then filled with water to the proper level, which is such that the meniscus in the capillary is at the lower end of the horizontal portion. The height of the column in the settling tube is then measured from the entrance of the side tube and also the volume. The stopcock at the upper end of the side tube is closed and the settling tube emptied. The suspension is then poured in up to the same level where the water stood and the tube placed in the thermostat. The tube is held firmly so that the angle of inclination of the side tube is constant. The stopcock is then opened and readings begun. The first

reading is taken 1 minute after setting the tube or at any other convenient interval. After about five readings have been made they are plotted and extrapolated to zero time in order to get the zero reading on the capillary tube. In the case of suspensions that settle slowly this is not necessary, as the reading at the end of 1 or even 2 minutes can be taken for the zero without introducing any appreciable error. In order to prevent the water from sticking in the capillary tube, it is recommended that a fairly large capillary (2 mm.) be used and also that some protective colloid, such as gum arabic, gelatin, saponin, etc., be added to the water in the capillary to reduce its surface tension and thus render it less liable to give false readings due to imperfections or specks of dirt in the capillary.

The tube as shown in Fig. 2 is not applicable in that form, because the water evaporates from both the large tube and the capillary, thus causing the recession to be more rapid than that due to the sedimentation alone. For that purpose a new tube (Fig. 4) has been designed (but not yet built).

The large tube, which should be about 2 cm. in diameter, has a ground, jointed cap, in the interior of which a small amount of water can be placed. This water is held at the same temperature as that in the tube proper, and hence by keeping the pressure of the water vapor constant below the cap any evaporation which takes place would naturally come from the water in the cap, as that is nearer the opening of the tube. The small bulb at the outer end of the capillary serves the same purpose.

The dimensions that should work best are given in Fig. 4, although the settling tube can be made any convenient length. Naturally, the longer the side tube the greater will be the initial level difference and hence the greater the accuracy, for a given suspension.

Preparation of the Sample

To get reliable results it is necessary that the sample be perfectly dispersed, or, in other words, all agglomerates must be broken up and only primary particles left. This may be done by moistening a weighed amount, for instance, 1 gram, on a glass plate and rubbing it with a spatula. A few cubic centimeters of a protective colloid solution, such as 5 per cent gum arabic, and a small amount of an electrolyte, such as 1 cc. of a 5 per cent barium chloride solution for barium sulfate or zinc chloride or sodium hydroxide for zinc oxide, etc., is added as a peptizing agent and the whole mixture rubbed well with the spatula. The mixture is then diluted gradually until it is fairly thin, washed into a graduate and made up to 100 cc., or whatever volume is desired. In this way a very good dispersion can be made.

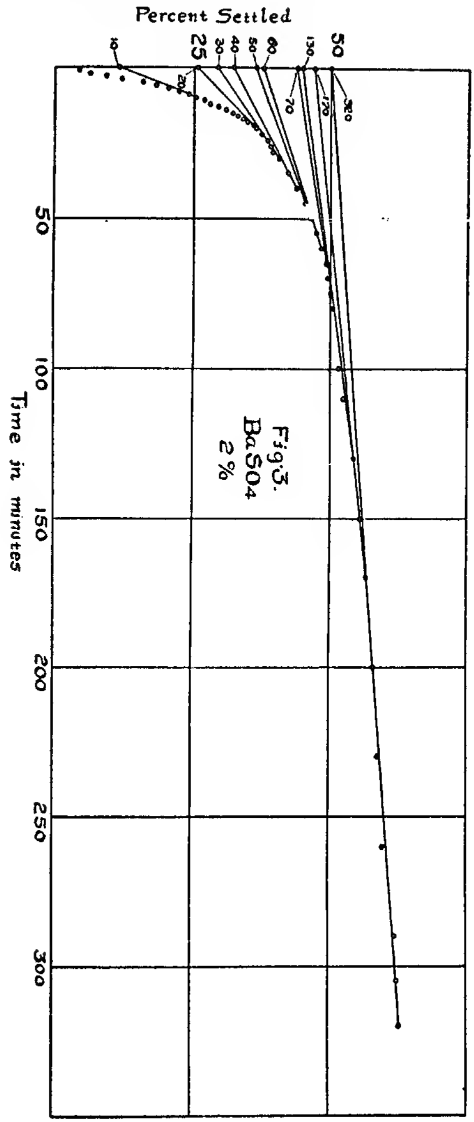

Percent Settled
25
50
Time in minutes
50
100
150
200
250
300
Fig. 3.
BaSO4
2%

FIG. 3.

TABLE I

Distribution of Particle Size in Suspension of Barium Sulfate

Time Minutes	Radius	Per cent
10	> 7.1μ	11.5
20	7.1 to 5.0	14.0
30	5.0 to 4.1	3.5
40	4.1 to 3.5	3.2
50	3.5 to 3.2	4.1
60	3.2 to 2.9	1.1
70	2.9 to 2.7	6.2
130	2.7 to 2.0	0.9
170	2.0 to 1.7	2.1
320	1.7 to 1.2	3.1
a	< 1.2	50.3

Results

Table I and Fig. 3 show the results obtained by this method on a 2 per cent suspension of barium sulfate. The curve is fairly smooth up to the point where 60 per cent of the material had settled out, at which point readings were stopped. If the experiment were carried much beyond this time, there would be some doubt as to the accuracy owing to the evaporation of the water in the settling tube.

The percentages of material of any given size range can be calculated from the sedimentation curve (Fig. 3). According to Svedberg and Rinde[4] the difference between the intercepts of any two tangents on the weight axis gives the amount of material having a size range as calculated from Stokes' law for the time interval chosen. In the present case the times are given in the first column of Table I and the radius range between two successive times is given in the second column. The percentages in the third column are obtained from the differences between the intercepts of the corresponding tangents.

In applying Stokes' law

$$r^2 = \frac{9}{2} \frac{\eta h}{(\Delta - \delta)gt}$$

h is measured from the surface of the liquid to the entrance of the side tube. Hence at any given time all particles of radius calculated from Stokes' law for this time will have reached the entrance of the side tube. By calculating the radii at successive time intervals the range of particle size settling between these time intervals is obtained. The figures given in Table I for the size range were calculated in this way. This table gives the distribution of the particle size for the particular sample of barium sulfate used.

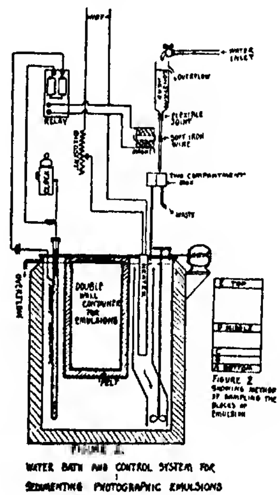
The Goodyear Tire and Rubber Co.,
Akron, Ohio.

[4] See also Svedberg, "Colloid Chemistry," A. C. S. Monograph Series, p. 144.

AN IMPROVED METHOD OF SEDIMENTARY ANALYSIS APPLIED TO PHOTOGRAPHIC EMULSIONS

BY F. F. RENWICK AND V. B. SEASE

In recent years, a great deal of attention has been given to the physical and photographic characteristics of the individual grains composing gelatino-silver-bromide emulsions. Svedberg's method of attacking the unsolved problems of the latent image by the statistical microscopic



FIGS. 1 and 2.

study of the behavior of plates coated with a single layer of grains has led to results of great importance and will doubtless continue to yield fruit, but it cannot be denied that it is extremely laborious and unsuited to the purposes of most industrial research laboratories.

The authors have, therefore, undertaken a study of photographic emulsions by sedimentation in the liquid state followed by a careful comparison of the physical, chemical, and photographic characteristics of the various layers.

In presenting the method, we desire to call attention to the first positive result of our work, namely, the demonstration of the fact that a photographic emulsion which contains both silver bromide and iodide consists, in many cases if not all, of grains which are by no means all alike in chemical composition but that, in the emulsions so far examined, the larger grains contain on the average a considerably greater percentage of iodide than the smaller ones.

If as seems most probable this should prove to be true of all iodobromide emulsions, it is clear that it must have an important bearing upon the relationship between grain size and sensitivity which so far

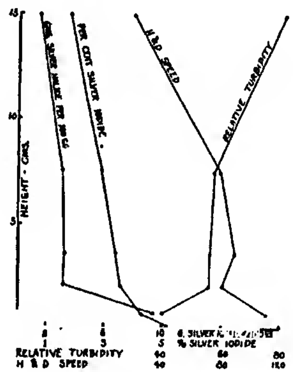


FIG. 3.

has met with no adequate explanation. In recent discussions of this question, the possibility of differences in chemical composition between the individual grains of an emulsion has been almost completely ignored.

The sedimentation apparatus is shown in Fig. I. It consisted of a large enameled iron container holding approximately 33 liters filled with water and surrounding a double jacketed vessel holding the emulsion. A sensitive thermoregulator controlled the operation of the temperature regulating mechanism. A fine steel needle, connected by a very light helical coil into the relay circuit, and suspended by a silk thread wound around a drum, was lowered by clockwork down the thermoregulator tube at such a rate that a very slow but perfectly regular fall of temperature amounting to 0.4° C. per hour resulted. The heating and cooling elements operated by the relay consisted of an electric heating element over which a vigorous circulation was maintained by pumping the water through a tubular jacket surrounding the heater in the manner shown in the figure and, further, when the relay operated to cut off the heater current, a tube carrying cold water

was deflected from a waste line to supply a small cooling stream of water to the bath.

The emulsion was thus brought slowly down to its setting point without appreciable disturbance by convection currents and after thorough chilling was cut up into a number of slices for complete examination. See Fig. 2.

The experimental data in Table I are given as an illustration of the method and the kind of information it is capable of furnishing.

TABLE I

SEDIMENTATION OF A MEDIUM SPEED NEGATIVE EMULSION

Layer	Mean Height in cms.	g. Ag. Hal. per 100 cc.	% AgI	Relative Turbidity	Relative Speed	6 Min. Gamma	Fog in 6 Min.
A	.7	9.36	4.34 *	39	110	1.10	.22
B	2.1	3.18	3.56	56	80	1.23	.10
C	3.5	3.24	3.44	56	88	1.09	.12
D	7.5	3.10	2.92	58	78	1.22	.12
E	15.0	1.50	1.82	82	16	1.76	.10
F †	—	3.32	3.20	54	65	1.14	.14

* Coarse grains settling out completely and adhering to bottom of crock gave 5.08% AgI.
† This sample represents a well stirred portion of the original emulsion kept in the same apparatus throughout the experiment.

These results are presented graphically in Fig. 3. Fig. 4 shows the grain size distribution of the original emulsion, and also of the top and bottom layers respectively after sedimentation.[1] These photomicrographs represent an emulsion having a large range of grain sizes.

We have also examined in this manner several examples of medium speed negative in which the range of grain sizes is small, and find that they resemble those just given for the large range emulsions, the results being perhaps less striking but no less definite. By the introduction of further refinements into the method, it is possible, as we shall show later, to effect a more complete separation between classes of nearly the same grain size. Suffice it to say at this point that we have been able even with these relatively uniform-grained emulsions to obtain bottom layers containing from 10 to 15 per cent more iodide than the top layers after sedimentation.

It may be well here to emphasize the fact that since chemical analysis can only give the average composition of the grains in any given layer, the extreme differences in composition between individual grains must certainly be much greater than the analyses indicate.

It is obvious that by repeated fractional sedimentation of suspensoids like these photographic emulsions, it will usually be possible to carry the separation of the different size classes much further than we have yet done. Unfortunately, the photographic characteristics of silver

[1] Magnification employed for all photomicrographs shown = 1000 diameters.

halide grains gradually undergo a change if an emulsion is kept molten at even a moderate temperature for long periods of time and, therefore, an analysis by this method becomes extremely difficult from the photographic standpoint. In order, however, to isolate the extreme grain

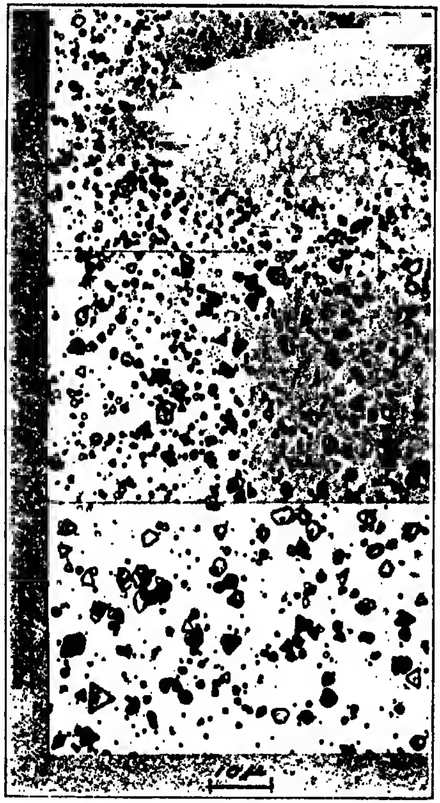


FIG. 4.

sizes present in one of these small range emulsions and to determine the average percentage of silver iodide in these extremes, we have carried through a few experiments in resedimentation, disregarding photographic characteristics. The original size distribution of one such emulsion is shown in the center of Fig. 5. Analysis showed the silver halides in this emulsion to contain 3.14 per cent silver iodide. The top and bottom layers of the first sedimentation were resedimented

separately, resulting in a separation of the grains into two extremes, shown also in Fig. 5, which were found to contain 2.61 per cent and 3.56 per cent of silver iodide, respectively. Another emulsion whose grains originally contained 4.13 per cent average silver iodide yielded

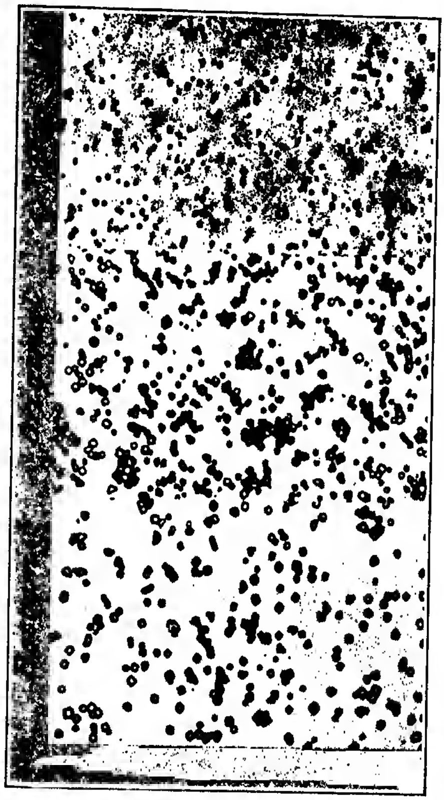


FIG. 5.

after a similar resedimentation two extreme grain size classes containing 3.89 per cent and 4.77 per cent of silver iodide in the top and bottom layers, respectively.

A modified procedure which results in a better separation at one operation of the various size classes of grains present is to dilute the emulsion to such a strength that it will only just set to a jelly when cool, and to allow its grains to sediment through a clear strong gelatin

solution of slightly higher specific gravity than the supernatant emulsion. We have found it better to run the strong gelatin solution slowly down through a narrow glass tube to the bottom of the vessel containing the diluted emulsion rather than attempt to pour the emulsion over the surface of the heavier gelatin layer. If done with care, the two layers are still very sharply defined by a great difference in their jelly strengths, even when more than 24 hours are taken for the cooling and setting operation. The objection to this method from the photographic standpoint lies in the fact that the environment of the grains under study has been changed, and that this may have resulted in alterations of their photographic properties. Provided, however, that the gelatin employed is free from desensitizing impurities, it is probable that such changes of properties of the emulsion will be due to the modified rate of diffusion of the developer rather than to fundamental changes in the sensitivities of the grains. In any case, it is not unlikely that a good deal of light may be thrown on exposure and development problems by studying the effects of such replacements of the suspending medium.

The variations in the average iodide content at various levels in the two types of emulsion discussed above when sedimented through clear gelatin are given in Table II.

TABLE II

SEDIMENTATION OF EMULSIONS INTO CLEAR GELATIN

Layer	Mean Height in cms.	Per cent Silver Iodide	
		Large Range Type	Small Range Type
A } Strong Gelatin	0.7	4.25	3.45
B } Strong Gelatin	2.1	4.00	3.41
C } Strong Gelatin	3.5	4.06	3.36
Gelatin Boundary	5.5	—	—
D } Weak Gelatin	7.5	2.32	3.35
E } Weak Gelatin	15.0	0.69	2.97

The original size distribution of the small range type of emulsion is shown in Fig. 6 along with the photomicrographs of the grains present in the top and bottom layers after sedimentation into clear gelatin. Fig. 7 shows the original size distribution and the top and bottom layers of the large range type of emulsion, after sedimentation in a similar manner.

The wide variety of grain sizes present in the bottom layer in Fig. 7, and many other similar experiments calls for discussion. It might, of course, be due to faulty technique, but we do not believe this can account for more than an insignificant proportion of it. The amount of original emulsion adhering to the walls of the vessel during the dis-

placement process is insignificant and inasmuch as the B and C layers showed an equally great variety of grain sizes, it cannot be explained on the assumption that all the larger grains in the emulsion had settled to the bottom followed by succeeding classes up to the smallest grains

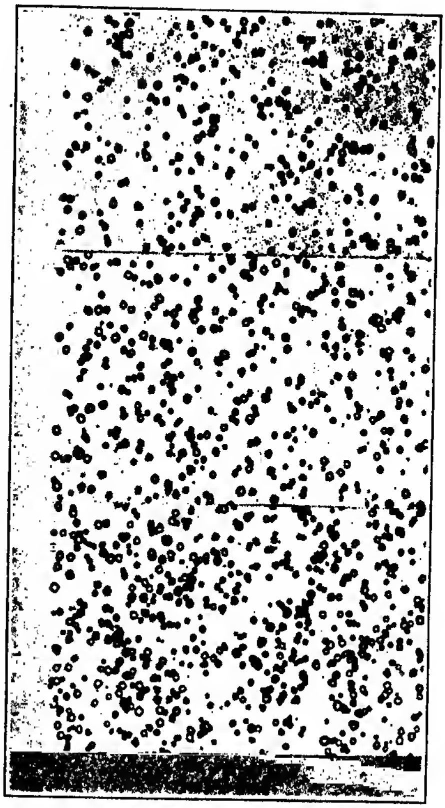


FIG. 6.

present. The possibility that the slight temperature gradient between the center and exterior of the cooling emulsion may give rise to noticeably unequal rates of sedimentation at different distances from the center has also been considered as an explanation of this phenomenon. A careful analytical, microscopic and photographic comparison of the core and outer annulus of the same layer in two cases lent however no support to this suspicion. We are, therefore, compelled to believe

that many of the small grains are capable of keeping pace with the larger ones in their descent.

Owing to the wide varieties of shapes present, it is obvious that Stokes' law does not apply. Many of the large grains are very thin

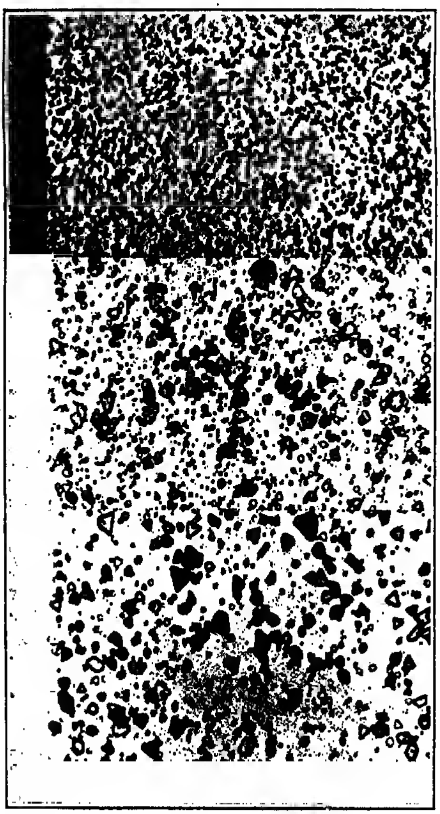


FIG. 7.

and offer considerable frictional resistance in falling through a viscous medium like gelatin, while the smallest grains are usually octahedra which present a very small surface in proportion to their mass. Another complicating factor is the difference between the specific gravities of silver bromide and silver iodide. It is important to note that although we have found the large grains to contain on the average the greatest percentage of silver iodide and to be present in the greatest numbers

in the bottom layers after sedimentation, this chemical separation is probably opposed by the specific gravity differences between the grains, for silver iodide is specifically lighter than silver bromide, the values being silver iodide 5.67, silver bromide 6.47. There may also be inclusions of gelatin and water within some of the grains which would lower their specific gravities relative to the rest. Lastly, it is even possible that local disturbance caused by the fall of the larger grains may cause some of the smaller ones to be carried down in their wake. In view of all these disturbing factors, it is not surprising that a clean cut separation of the various size classes by sedimentary analysis seems impossible in the case of silver iodobromide emulsions in gelatin.

The object of this paper is not so much to invite discussion of the photographic data it contains, since these are merely preliminary in character, as to call attention to the possibilities which we believe are afforded by the method of sedimentation into a slowly congealing medium.

It will be obvious that other media than gelatin might be employed where desired, for instance, molten paraffin or vaseline, or a medium like cellulose xanthate (which slowly gels on standing, especially with a slowly rising temperature, and can be restored to solution by dilute alkali).

Any such method has two positive advantages over the more usual one of drawing off liquid samples of the suspension at different levels. In the first place, sedimentation can be allowed to proceed undisturbed for any desired period and examination can be deferred to any convenient opportunity after the gel has formed. Secondly, the gel can be conveniently cut up for analysis both chemically and microscopically in any desired direction, whereas the drawing off of liquid samples is very liable to disturb the sedimentation process and the portion withdrawn is automatically reblended. While, therefore, the method is probably somewhat less expeditious, it will, we believe, often be found more convenient and more certain than the customary procedure and appears worthy of trial in other fields than the limited one in which we have applied it.

E. I. du Pont de Nemours & Co.,
Redpath Laboratory, Parlin, N. J.

SOLS WITH NON-SPHERICAL PARTICLES

By Herbert Freundlich

Until a few years ago the structure and form of a colloidal particle had received but little attention from investigators and the conceptions in this regard were of a general nature, such that the particles are amorphous and accordingly spherical, or to the contrary, that they are crystalline throughout. Only a few isolated observations of a specific nature had been made, as for instance the observation by Zsigmondy and Bachmann [1] of threadlike particles, which are found in soap solutions. Furthermore it was known that iron oxide sol exhibited the so-called Majorana phenomenon,[2] that is, double refraction when subjected to the influence of a magnetic field, which was explained by a definite structure of the colloidal particles and their orientation in the magnetic field. This explanation could not be confirmed by experiment because the iron oxide sol cannot be analyzed in the ultramicroscope. As is often the case, a clear understanding of this subject was first furnished by an especially outstanding case of a colloidal solution with non-spherical particles, which enabled the correlation of the innumerable phenomena that are associated with these sols.

This sol is a colloidal solution of vanadium pentoxide [3] and is easily prepared as follows: ammonium vanadate is ground with dilute hydrochloric acid and the flocculent precipitate of vanadium pentoxide which forms is removed from the liquid by filtration, and washed with water until it begins to pass through the filter. The precipitate is now dispersed by shaking it vigorously with a suitable quantity of water. The sol is a clear liquid of a beautiful reddish-brown color. When freshly prepared the sol gives as a rule no indications that the colloidal particles are non-spherical; only after the sol has aged do the properties which are due to a definite structure of the particles gradually become more and more prominent. If the sol is old enough rod-like particles possessing a slow Brownian movement can be directly observed with the ultramicroscope. Longitudinally the particles are of microscopic dimensions, often more than 1 μ long, but their lateral dimensions remain ultramicroscopic, or amicronic, and therefore the particles cannot be seen in the microscope. If the particles are still smaller, they are characterized by the fact that under the ultramicroscope they exhibit pronounced *scintillations* and not a continuous radiation of light, as in the case of a sol with spherical particles, as for example a sulphur sol.

The cause of these scintillations has already been determined by Siedentopf [4] in his microscopic researches, and the same considerations can be applied to the behavior in the ultramicroscope.[5] An elongated particle can be seen in the ultramicroscope only when the light rays strike the particle perpendicular to its longitudinal axis and when the particle lies in the plane of observation of the microscope. If the light strikes the particle in a direction parallel to its longitudinal axis or if the particle is perpendicular to the plane of observation it cannot be seen or in any case much less distinctly. Since the particles have a Brownian movement, they become luminous when in the correct position, and disappear in all other positions, and this gives rise to scintillations.

The vanadium pentoxide sol exhibited the *Majorana phenomenon.* When a trough containing the sol is so placed in a magnetic field that the illuminating light rays pass through the liquid layer perpendicular to the lines of force, the layer behaves like the lamina of a uniaxial crystal which had been cut out parallel to its axis and whose axis lay in the direction of the lines of force. That is, the sol showed the characteristic double refraction in the magnetic field. A strong illumination was observed through crossed Nikol prisms when in a horizontal magnetic field the electrical vector of the illuminating rays was inclined at an angle of 45° to the lines of force. This phenomenon has long been explained in the case of iron oxide sol by an orientation of particles of definite structure in the magnetic field. Furthermore it developed that this orientation can be caused by other forces. The magnetic field could be replaced by an electric field. A layer of sol through which a direct current was flowing behaved with respect to double refraction in a similar manner to the lamina of a uniaxial crystal, whose optical axis coincided with the direction of the current. The effect produced by an alternating current was the same as that produced by a direct current. This is in agreement with the experimental fact that colloidal particles have an oscillatory motion in an alternating electric field since they respond directly to the current pulsations.[6] It is therefore evident that elongated particles can be oriented by an alternating current as well as a direct current in such a way that their longitudinal axes will assume the direction of the current. The orientation of the particles can be effected by a simpler method as for instance merely by subjecting the liquid to mechanical flow. Due to the friction between the adjacent lines of flow moving at different rates, the rod-like particles assume a direction practically parallel to the lines of flow, and disc-like particles are ordinated so that their surface lies in a plane of flow of uniform velocity. When the vanadium pentoxide sol is caused to flow through a tube with parallel walls it again exhibits double refraction similar to the lamina of a uniaxial crystal which has been cut parallel to its axis and whose axis lies in the direction of flow. A strong illumination is observed through crossed Nikol prisms if the

electrical vector of the illuminating rays is inclined at an angle of 45° to the perpendicular direction of flow. Since in the case of vanadium pentoxide sol, in contrast to the iron oxide sol, the particles are often large enough to be seen under the ultramicroscope, it could be directly determined whether the particles are oriented in the manner assumed abeve.[7] In accordance with the above mentioned optical behavior, which gives rise to scintillations only those particles can be seen in the slitultramicroscope which lie in a plane approximately perpendicular to the illuminating rays in the plane of observation. If an electric current is passed through the sol under the ultramicroscope in a direction parallel to that of the light rays, then the particles disappear and the observation field darkens. The particles now lie with their axes parallel to the direction of the current and the light rays strike the particles parallel to their axes. Consequently no light is diffracted and the particles cannot be seen. If the current circuit is opened, through the influence of the Brownian movement the particles assume their original state in about two minutes.

The same experiments could be repeated with the iron oxide sol.[8] Not only was the sol double refracting under the influence of a magnetic field, but also under the influence of an electric field, and when subjected to flow. This showed that the Majorana phenomenon is but a special case of a more general group of phenomena, which are observed with sols containing non-spherical particles, when subjected to influences that effect a definite orientation of the particles.

It is surprising that the double refraction is noticeable in such extreme dilutions. In the case of vanadium pentoxide sol Zocher[9] was able to prove the existence of double refraction in a solution whose concentration was only 1/30 milligram per litre. The color on the other hand was no longer apparent in a solution of ten times this strength. This becomes apparent when one considers that the color is dependent upon a small change in the penetrating light, while the double refraction makes itself apparent as an illumination between crossed Nikol prisms, and depends upon the action of the total substance.

For the sake of simplicity so far only the double refraction of sols has been discussed. But this is not the only change in optical behavior which the sols suffer when subjected to flow or to the influence of a magnetic or an electric field. The iron oxide sol becomes slightly positive *dichroic* and the vanadium pentoxide sol strongly positive *dichroic* when its particles are oriented.[10] The extraordinary ray which vibrates parallel to the longitudinal axes of the particles and hence in the direction of flow is more strongly absorbed than the ordinary ray, which vibrates perpendicular to the longitudinal axes and the direction of flow. The orientation of the particles is also observed in the case of the Tyndall phenomenon. The above referred to optical conditions, which give rise to scintillations of the particles under the ultramicro-

scope, must also manifest themselves in the *Tyndall light*, as soon as the particles assume a definite position with respect to the illuminating rays, when subjected to flow or to the influence of a magnetic or an electric field. The diffraction of light is strongest when the elongated particles are in a position perpendicular to the direction of the illuminating rays in the plane of observation. In every other position the particles diffract the light much less or not at all. This phenomenon is designated by Zocher [11] as *dityndallism* or double diffraction. The extraordinary *streaks* [12] which are observed when a sol, containing non-spherical particles, is stirred are due to dityndallism. When an older vanadium pentoxide sol is stirred, the liquid fills up with yellow streaks of a beautiful silky luster which disappear when the liquid comes to rest. When the liquid is stirred eddy currents in different directions are set up and the particles carried along in them send out a stronger or weaker Tyndall light, depending upon their position with respect to the illuminating rays and the observer. The occurrence of these streaks is the simplest means of determining whether a sol contains spherical or non-spherical particles.

A number of the most important properties which are often associated with sols possessing non-spherical particles have here been described. I do not wish to go into details with regard to certain other properties but shall touch upon them by discussing a few fundamental questions, which one is inclined to ask. The first of these is: What state of aggregation do the particles of such a sol possess, to which the answer is that the particles in the majority of cases are crystalline. This could be established in the case of vanadium pentoxide and iron oxide sols.[13] If the colloidal particles are filtered off with an ultrafilter and investigated by means of x-rays according to the method of Debye and Scherrer, distinct interference lines are obtained which are slightly broader than those of coarse crystalline substances. It has been shown that the particles of many sols, such as those of the colloidal solution of gold, silver, aluminum hydroxide, etc., are crystalline, while the precipitates of aluminum hydroxide or iron hydroxide which have been suddenly precipitated from solution are amorphous. According to Haber [14] this is due to a competition between a *grouping velocity* and an *orientation velocity*. If the period of precipitate formation is very short, the tendency for definite orientation of the molecules is not allowed enough time to assert itself, the particles remain in a state of disorder and the precipitate is amorphous. If on the other hand more time is available as in the case of aluminum hydroxide or iron hydroxide sols formed by hydrolysis the process of orientation can take place and the particles are crystalline provided that the tendency to crystallize is great enough. In many sols, as for example those of cerium hydroxide, zirconium hydroxide, and thorium hydroxide this tendency is expressed to such a small degree that the particles of freshly prepared

sols are amorphous and become crystalline only after a long period of time. These sols exhibit none of the properties of sols with non-spherical particles. The particles are probably to a large extent spherical. Furthermore there exist colloidal solutions whose particles deviate from the spherical form although they are not crystalline. It would lead too far to go into details of these peculiar cases here.

Although the particles of many sols with non-spherical particles are crystalline, it does not follow that the ageing of the sols, which leads to those characteristic phenomena, depends exclusively upon variations which are due to a crystallation procedure; in many cases it is more a question of coagulation. This is evident in the case of various dyestuff sols which are pronounced examples of sols with non-spherical particles; amongst these are solutions of benzopurpurin and cotton yellow.[15] Concentrated aqueous solutions of these dyestuffs show a far-reaching similarity to the solution of vanadium pentoxide. They display double defraction, dichroism, dityndallism, show streaks, and rod-like particles under the ultramicroscope. Dilute solutions certainly do not become double refracting in the course of a long time. If electrolytes are now added to these dilute solutions in a concentration which is one tenth that required to cause coagulation in the form of a pronounced turbidity, then in the course of time double refraction is noticeable.[16] This process follows completely the laws of coagulation; since the dyestuff particles are negatively charged the active ions are the cations and their effect is governed by their valence and adsorption. That is, a much smaller concentration of a bivalent cation than a univalent cation will produce double refraction in a given period of time. Hydrophil colloids, as for example gelatin, have a protective effect. By heating to a suitable temperature the double refraction can be caused to disappear and this process is in all respects similar to peptization. One gains the impression that *directed coagulation* which is determined by an orientation velocity must be differentiated from *undirected coagulation* which depends largely upon the grouping velocity. This is shown most clearly by the following experiment.[17] When an electrolyte is added, as explained above, to a benzopurpurin solution in small concentrations the solution exhibits double refraction. If on the other hand a large concentration of electrolyte is added to produce rapid coagulation and the flocks peptized by washing with water, a sol is obtained which is similar to the original sol in that it does not exhibit double refraction. This can hardly be explained otherwise, than that in the case of rapid coagulation with large electrolyte concentration the particles in the flocks lie completely in disorder and when peptized remain so, while in the case of slow coagulation with small electrolyte concentrations they form particles of definite structure which give rise to the double refraction.

It cannot be determined from the foregoing experiments whether the

occurrence and growth of the anisotropic properties of vanadium pentoxide sol are due to coagulation or to a growth of the particles through crystallization. The ageing of these sols was pursued by quantitatively determining the double refraction and dichroism when subjected to flow.[18] It developed amongst other things that foreign substances which are present in the ammonium vanadate have a strong influence on the velocity of ageing. Thus arsenic acid seems to have the effect of considerably decreasing the velocity of ageing. This explains why the time which is necessary for the sols to become double refracting was found to be so different by various observers. In the case of such influences one is inclined to think of a decrease in the velocity of crystallization rather than a decrease in the velocity of coagulation. On the other hand a more theoretical consideration indicates that it is more a question of gradual coagulation. Errera[19] discovered the peculiar phenomenon that the dielectric constant of a vanadium pentoxide sol, when determined according to the method of Nernst, increases, together with the double refraction, strongly with age. In the case of a freshly prepared sol the value of the dielectric constant is approximately equal to that of water about 80, with an older one he observed a value as high as 400. This remarkable behavior is explained by Szegvari and Wigner[20] as follows: Under the influence of the alternating current which is used in the above method, the particles become oriented so that their longitudinal axes lie in the direction of the current; there occurs a displacement of the two layers of the double layer which surrounds the particle. This causes an increase in the capacity and accordingly an increase of the dielectric constant of the sol. Now according to the theory of these phenomena the dielectric constant increases with the ratio of the length of the particles to their thickness. But their ratio should be constant for a given crystallization process, since the different surfaces of a crystal grow with a constant speed of crystallization. Therefore one would not understand from the point of view of crystallization velocity why the values of the dielectric constant increase so strongly in the course of time. In the case of a directed coagulation however, the particles could join end to end and hence cause a stronger variation of the relation length to thickness.

A second question is: What kind of double refraction do these sols exhibit? Is it a *rod-like double refraction* or does it depend upon the *double refraction of the individual colloidal particles?* As we know, optical isotropic particles which are not in themselves double refracting also yield double refracting structures, provided they have an elongated form and are regularly oriented. This type of double refraction is termed rod-like double refraction and the theory involved has been developed largely by O. Wiener.[21] Two results of the theory are important here. First, the degree of double refraction is dependent

upon the difference between the indices of refraction of the particles and the surrounding medium, and when this difference is zero, the double refraction is zero. Secondly, the rod-like double refraction must always be positive. Examples of rod-like double refraction are known, as for example the case of fibre-alumine,[22] and, as recently shown by Sueffert and Zocher,[23] the scales of butterflies' wings. It would be quite conceivable that the double refraction of these sols is dependent upon a rod-like double refraction. Experience has shown that this is not the case or in any event only to a small degree. Of course this question cannot be readily tested experimentally. It would be necessary to supplant the aqueous dispersing medium by a dispersing

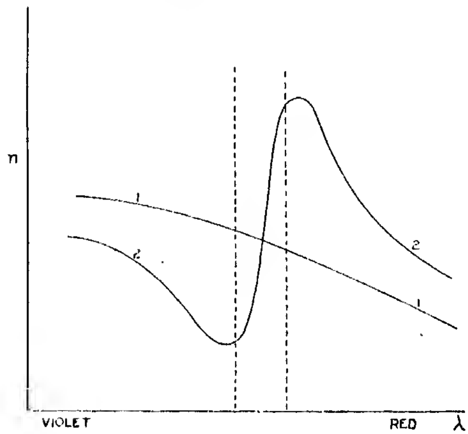


FIG. 1.

medium having an index of refraction equal to that of the particles; should the double refraction disappear it would indicate rod-like double refraction. It developed however that various sols such as benzopurpurin sol exhibit negative double refraction in contradiction to the theory of rod-like double refraction. In general the degree of double refraction is also greater than that which would be expected from rod-like double refraction alone. As a rule the double refraction of these sols is due to the crystalline particles which are themselves double refracting. When the particles in the liquid are moving in a state of disorder, double refraction cannot be detected but is noticeable when they are regularly oriented by appropriate means.

A relation exists between the double refraction and the dichroism of these sols which according to the recent researches of Zocher[24] correlate in general double refraction and dichroism of anisotropic structures. For the sake of simplicity it is assumed that the dichroism

is so pronounced that the extraordinary ray of the sol is not appreciably absorbed while the ordinary ray is strongly absorbed. This leads to a negative dichroism and let us assume that the absorption lies in the green portion of the spectrum. Between the index of refraction and the wave length of the extraordinary ray there exists a simple relation as shown by curve 1, Fig. 1. The indices of refraction n are plotted as ordinates and the wave-lengths λ as abscissæ. For the ordinary ray on the other hand we have curve 2, representing the well known influence of absorption on dispersion. This need not always be the course of the two curves but it is a very probable one. Positive double refraction is observed when the index of refraction of the extraordinary ray is greater than that of the ordinary ray, and negative double refraction for the reverse case. It is now evident from Fig. 1 that the double refraction in the blue and violet must be positive and negative in the red. This complicated behavior was actually observed by Zocher[25] in the case of an anilin blue sol with non-spherical particles. All the characteristics of the above described examples are met with in this sol: Strong absorption in the green, negative dichroism, negative double refraction in the red, positive in the blue. The relationships in Table I could be confirmed in a general way from the anisotropic sols.

TABLE I

Negative Dichroism	*Double Refraction*
Absorption range in middle of spectrum	*negative* in *red*, *positive* in *blue*.
Absorption range in *red* end of spectrum	*positive* in remainder of spectrum.
Absorption range in *blue* end of spectrum	*negative* in remainder of spectrum.
Positive Dichroism	*Double Refraction*
Absorption range in middle of spectrum	*positive* in *red*, *negative* in *blue*.
Absorption range in *red* end of spectrum	*negative* in remainder of spectrum.
Absorption range in *blue* end of spectrum	*positive* in remainder of spectrum.

In the presence of more absorption bands the conditions can become even more complicated, but this will not be discussed in detail here.

A third question may be discussed here: Are the anisotropic properties of these sols due to the coarse submicronic particles or the smaller amicronic particles? The question arises because the Wiener theory of rod-like double refraction requires that the particles as well as the distances between them must be small as compared to the wave length of light. Weigert[26] is therefore of the opinion that also in the case of sols with non-spherical particles the anisotropic properties depend largely upon the amicrones and less upon the particles which can be seen in the ultramicroscope. Of course with these sols a rod-like double refraction is not involved, and the conclusion is therefore not so binding as it otherwise would be.

But other experimental facts indicate that not only is it essential that the individual larger particles of these sols be oriented, but that there are swarms of smaller oriented particles, and these are perhaps especially important in determining the behavior of the sols. Such swarms can be recognized directly under the ultramicroscope, if the latter is equipped with diaphragms suitable for the study of such sols with non-spherical particles. The use of these diaphragms an *azimuth* and an *aperture diaphragm,* in the ultramicroscope was introduced by Szegvari [27] after Siedentopf [28] had already applied the azimuth diaphragm in microscopic researches. The applicability of the azimuth diaphragm depends upon that phenomenon, which as discussed above, gives rise to scintillations of the particles, that is, the elongated particles

FIG. 2.
Without azimuth diaphragm.

FIG. 3.
With azimuth diaphragm.

FIG. 4.
With azimuth diaphragm.

are visible in the ultramicroscope only when the light strikes them perpendicular to the longitudinal axes and when they lie in the plane of the observer. In the slit ultramicroscope the light always strikes in a definite direction and this cannot be changed at will. In the Kardioid ultramicroscope this can be attained however. In this instrument the light rays approach from all sides. If an azimuth diaphragm is introduced under the condenser, that is a movable slit diaphragm, through which the slit goes diametrically, then by turning the diaphragm the light can be made to strike the ultramicroscopic preparation from any desired direction. (Although the details will not be discussed here, Szegvari has shown that in the use of the azimuth diaphragm a suitable aperture must be employed. He therefore always employed the azimuth diaphragm together with a suitable aperture diaphragm, an iris diaphragm, which enabled the correct setting of the aperture.) If an old vanadium pentoxide sol is examined with the Kardioid ultramicroscope and the azimuth and aperture diaphragms, the results, which are shown in Fig. 2-4 are obtained. Without the azimuth diaphragm (Fig. 2) particles possessing all possible directions are seen, but with

the azimuth diaphragm (Fig. 3 and 4) only those particles can be recognized which lie perpendicular to the illuminating beam. These are partly arranged in cloud-like swarms. It is noticed that certain spots, which are light in Fig. 3, appear completely black in Fig. 4. These clouds consist of very small colloidal particles, which cannot be seen in the ultramicroscope, but whose scintillations are still just noticeable. These swarms have an unquestionable significance concerning the behavior of sols with non-spherical particles, they are oriented by flow and also change their structure under proper conditions. Old concentrated vanadium pentoxide sols often exhibit a permanent spotted double refraction,[29] similar to that found in the melts of anisotropic liquids. This depends upon the presence of such swarms. Furthermore a spotted illumination is visible between the crossed Nikol prisms in a polarizing microscope, which is naturally caused by the swarms. It must be emphasized here that the sol as examined in figures 2-4 was not coagulated; the coagulated sol gave an altogether different picture.

By the use of these diaphragms several more very remarkable advancements were made, of which two examples will be mentioned. First by using the azimuth diaphragm the scintillation of non-spherical particles is more pronounced than when not using the diaphragm. This is due to the fact that by the use of a narrow azimuth, non-spherical particles illuminate only in a small portion of the field. By the use of a narrow slit and a very small aperture it was possible to show that the iron oxide sol, which cannot be seen in the ultramicroscope, exhibits pronounced scintillations.[30] It follows from this that its particles are non-spherical and the assumption of its non-spherical form which had been made to explain the Majorana phenomenon is herewith proven. Ordinated coagulation can be proven to a large degree by the use of azimuth diaphragms.[31] If one observes an elongated particle of such a sol while at rest with the aid of the azimuth diaphragm and then turns the diaphragm slowly, the whole particle disappears simultaneously if it is composed of a single uniform flat crystal. It is often noticed, however, that the whole length of the crystal does not disappear uniformly but that it presents a broken line. The picture is separated into several parts which disappear when the diaphragm is turned further. This indicates that the particles are not uniform throughout but are composed of a number of small particles linked together. This phenomenon is easily observed in the case of benzopurpurin sols containing electrolyte, for which directed coagulation had been assumed above.

The above discussion is primarily of theoretical importance. There is all the reason to assume that many natural sols as well as those of technical importance contain non-spherical particles and that this is important in determining their behavior. This is already known in the case of fibrin sols containing decidedly rod-like particles.[32] Many

gels also exhibit this phenomenon, as for example, those of lithium ureate[33] as well as various salts of quinine and its derivatives.[34] I *might remind you that in the cellulose fibers of wood, cotton, and other fibers, it is found in examinations* by the roentgen ray method of Debye and Scherrer that crystalline particles are arranged about an axis symmetrically.[35] By the same method it was proven that many fibrils, sinews, and muscle fibers are not strictly amorphous.[36] Elongated particles could be detected in connective tissues by the aid of the azimuth diaphragm.[37] Non-spherical particles are therefore in all probability the ultimate units of many biological structures.

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STUDIES WITH THE KINOULTRAMICROSCOPE

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In the introduction to his most recent book,[1] Svedberg writes, "The central point in colloid sols and gels is the particle—like the molecule in chemistry and the cell in biology." It is the constant aim of the colloid chemist and physicist to explain and interpret the behavior of such systems in terms of the particle and its relation to its environment. Any means, therefore, which allows the scientist to perceive the individual particles in a disperse system and the changes which they undergo provides him with a most valuable tool. It is for this reason that ultramicroscopic methods of study have played such an important rôle in the development of colloid physics and chemistry into an exact science. The influence of this typical colloid method of study has been felt outside the bounds of colloid science also. Thus a great deal of the most direct experimental data in confirmation of the molecular-kinetic theory of matter has resulted from the quantitative study of the Brownian movements. The Brownian movements and related phenomena have furnished exemplary data upon which the predictions of the theory of probabilities and statistics could be tested. A microcosmos has been revealed by such studies, with natural laws that are in part quite different than those which are met in the macrocosmos in which we live. Likewise the data on such phenomena as coagulation, cataphoresis and the charge on the particle which may be interpreted with greatest certainty in terms of the individual particles have been obtained by means of microscopic methods of study.

The period of usefulness of ultramicroscopic methods has by no means been passed. In this preliminary paper, the attempt will be made to show further examples where they may be used to advantage, especially combined with the cinematograph to form what we may call for convenience a kinoultramicroscope. Such a combination possesses several advantages. In the first place, quantitative studies with the ultramicroscope frequently involve the use of statistical methods. Visual observation over long periods of time to allow the accumulation of sufficient data is very fatiguing and is unusually endangered by subjective errors; in the "kino" method, a permanent objective record is obtained which may be analyzed at will. In the second place, the continuous and extremely intense illumination incident

[1] "Colloid Chemistry," A. C. S. Monograph [1924].

to visual observation of the system under examination offers a very serious problem in the maintenance of a uniform and constant temperature in the system. With the "kino" method, the specimen may be protected from the illumination except during the actual photographic exposures, which may in general be separated by relatively long intervals. Thirdly, in the case of processes or changes which take place rather rapidly the "kino" method is the only one available for procuring during the course of the process data in sufficient quantity to justify the application of statistical analysis. Of course it must be admitted that the kinoultramicroscope can be used only when the photographic process is sensitive enough to record the data desired. This places a limit upon the smallness of the colloid particles which may be photographed and the speed of the process which may be followed.

I shall not discuss in this connection the previous use of the cinematograph in connection with the ultramicroscope,[2] but shall limit myself to those cases for which my films may serve as illustrations.

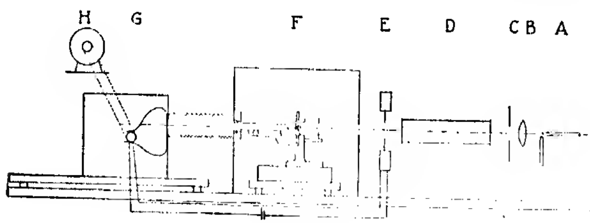

FIG. 1.

APPARATUS

The general arrangement of the apparatus is schematically illustrated in Figure 1. *A* is a 15 ampere d.c. arc with vertical and horizontal adjustments. The rays of the arc are made slightly convergent by a wide aperture, short focus quartz lens *B*, and then freed from heat rays by a 25 centimeter layer of water or alum solution in the chamber *D*. E is a photographic shutter operated by means of an electromagnet the excitation of which is controlled at the camera. At *F* is a Zeiss microscope provided with a Cardioid condenser, apochromatic objective ($f = 3$ mm.) and compensating or projection oculars. The microscope is protected by means of a house from which a bellows leads to the standard Universal cinematograph *G*. With the special constant speed motor *H* driving the camera, a series of pictures may be taken with a

[2] By means of a semi-cinematographic arrangement (moving photographic plate) described by Svedberg in his book, "Die Existenz der Moleküle," 187, Leipzig, 1912, Nordlund, *Z. physik. Chem.*, 87, 40 (1914), photographed the Brownian motion of single mercury particles. Lorenz and Eitel, *Z. anorg. Chem.*, 87, 357 (1914), thus recorded the spontaneous fluctuations in concentration of smoke particles suspended in air. In their studies of the ultramicroscopic structure of soaps, the cinematograph was used by Darke, McBain, and Salmon, *Proc. Roy. Soc., London*, 98A, 395 (1921).

constant and known time interval (normally ½-2 seconds). The entire apparatus is mounted in such a way as to minimize vibrations. For magnifications of 300-500 X, exposures are usually 1/50-1/5 seconds.

SIZE AND DISTRIBUTION OF SIZES OF PARTICLES

The simplest and most direct application of the kinoultramicroscope has for its object the determination of the sizes of the particles in a colloid solution. The uniform motion of a sphere through a viscous medium is described by the resistance law $f = 6\pi\eta rv$. If the sphere is

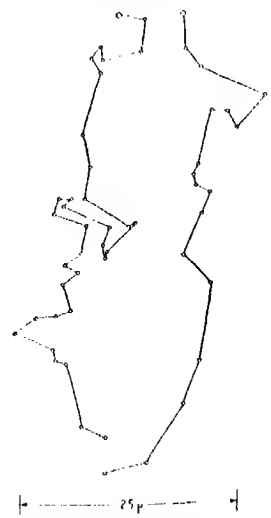

FIG. 2.—Paths of falling mercury particles showing superposed Brownian motion.

moving under the influence of gravity, the motion is described by the well known Stokes' formula $r^2 = \frac{9}{2}\frac{\eta v}{(d_p - d_m)g}$. The determination of the effective radius of the particles in a sol therefore depends upon the determination of the rate of rise or fall of the particles. The rate of rise or fall may be readily recorded with the kinoultramicroscope in a series of pictures at constant, known intervals of time. Projection of the pictures upon a calibrated screen allows the measurements to be made in a simple and convenient manner. Figure 2 is a composite of a series of pictures at intervals of 2.05 seconds showing the motion of mercury particles prepared by electrical pulverization with the Tesla coil.[8] Since the Brownian motion is superimposed upon the vertical

[8] *J. Am. Chem. Soc.*, Sept., 1924.

component due to gravity, the size determination requires the measurement of the rate of fall or rise over a number of intervals of time in order that the average obtained may represent the influence of gravity alone (for motions in both directions along the vertical axis are equally probable as far as the Brownian motion is concerned). A more accurate idea concerning the degree of dispersion of a sol is obtained by measuring in the fashion outlined, the sizes of a large number of particles, and constructing a distribution curve.

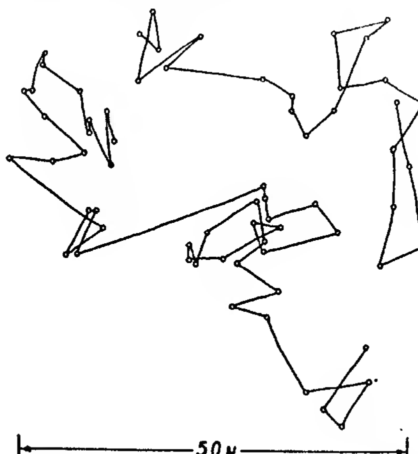

FIG. 3.—Brownian motion of the particles in a sulfur sol.

If the particles in a sol are too small to show an appreciable sedimentation under the influence of gravity, a study of the Brownian motion may be used to arrive at the effective radius of the particles. This procedure also is based upon the application of the resistance law already stated. For spherical particles moving in a homogeneous viscous medium, the Brownian motion is described by the "displacement law"—the mean square of the displacements along any axis during constant intervals of time is equal to the product of twice the diffusion constant by the time interval, or $\overline{\Delta_x^2} = 2Dt = \frac{RT}{N}\frac{t}{3\pi\eta r}$. Since the diffusion constant is dependent upon the effective radius of the particle, the determination of the average square displacement furnishes a means of measuring the effective radius. Of course, because of the limits imposed by the insensitivity of the photographic process, very small particles cannot be studied in this way. Figure 3 is a composite showing the motion of sulfur particles (Odén's sol) the density of which is too small to lead to sufficiently rapid sedimentation.

Theoretically, the distribution of size of particles could be determined by studying the Brownian motion of a large number of particles. But since the adequate determination of a single mean square displacement requires the measurement of a large number of pictures, the labor necessary for obtaining a distribution curve, even with the "kino" method is practically prohibitive.

Verification of Theories of Probabilities and Molecular Statistics

I have already referred to the important rôle which the ultramicroscope has played in the experimental testing of the theories of probabilities and molecular statistics. Combined with the kinetic theory, the labors of Einstein and Smoluchowski have been eminently successful in dealing with changes which may take place in a system of discrete particles in unordered and chaotic motion. These studies have been of great significance, not only as a portion of colloid science, but of greater importance in connection with theoretical physics and chemistry. I need only remind you of the influence of these studies upon our views of reversibility and irreversibility by making more accurate our conception of the second law of thermodynamics and showing us the limits of validity of this empirical law. The most direct confirmation of these theoretical investigations have resulted from ultramicroscopic studies of the Brownian motion and related phenomena.

There has been a lull in investigations of this kind. On the whole, scientists have considered the experimental confirmation adequate and the theories have gained general acceptance. On account of the untimely death of the Polish pioneer, Maryan Smoluchowski, no significant theoretical advances have been made recently in the field of colloid statistics. But as I shall indicate, the accepted theories may be made useful in an indirect way as in the study of gel structure.[4] Before dealing further with the question of gel structure, I may call attention to a few of the theoretical results which may be useful.

Smoluchowski has evaluated not only the average square displacement, but also the probability for the occurrence of a displacement of any magnitude, *i.e.*, he has given the equation for the magnitude-distribution of the displacements which a colloid particle should undergo. The probability W_x for a displacement along the x axis between x and $x+dx$ in magnitude is given by

$$\text{Eq. 1} \quad W_x dx = \frac{1}{2\sqrt{\pi D t}} e^{-x^2/4Dt} \, \mathrm{dx} = \frac{1}{\sqrt{2\pi \overline{\Delta}_x^2}} e^{-x^2/2\overline{\Delta}_x^2} dx.$$

[4] Prof. The Svedberg made such a suggestion originally in a paper presented before the First National Symposium in Colloid Chemistry, University of Wisconsin, June [1928], and in "Colloid Chemistry," A. C. S. Monograph [1924], p. 93. These studies were begun upon the suggestion and with the helpful advice of Professor Svedberg.

The theoretical investigations consider not only the life history of a single particle, but also describe on a statistical basis the spontaneous changes in concentration which take place in a very small volume of a sol cut out geometrically from a larger volume. Perhaps on the basis of the average concentration of the sol, there should be 3 particles in the element of volume. But as a matter of fact, and contrary to older ideas of reversibility and irreversibility and the laws of thermodynamics, the number of particles present in the volume at any moment may vary from zero upwards. (A film record was projected showing the spontaneous fluctuations in a gold sol.)

The normal distribution curve showing the frequency of occurrence of any given number of particles in the given volume is given by the equation

Eq. 2 $$W_n = \frac{e^{-\nu}\nu^n}{n}$$

in which W_n is the probability for the occurrence of n particles in the volume in which the average is ν. The momentary degree of fluctuation is equal to $\delta = \frac{n-\nu}{\nu}$, and the average fluctuation is

Eq. 3 $$\bar{\delta} = \frac{2\nu^k e^{-\nu}}{k!}$$

k being the largest whole number not exceeding ν and the mean square of the fluctuations is

Eq. 4 $$\bar{\delta^2} = \frac{1}{\nu}$$

Likewise the speed of the fluctuations has been evaluated. The average change in the number of particles during the time t starting from an original concentration of n particles is

Eq. 5 $$\bar{\Delta}_n = (\nu - n)P$$

and the mean square change from any original state is

Eq. 6 $$\bar{\Delta^2} = 2\nu P$$

where P is a diffusion factor giving the probability that a particle originally anywhere in the element of volume v will at the end of the time t be outside of this volume. There are still other quantities given by the application of the theory of probabilities to spontaneous concentration fluctuations, but these will suffice to show how they may be used. The essential point is that these various equations are valid only if the medium in which the particles are moving is homogeneous with

relation to its influence upon the motions of the suspended particles. That is, the frictional resistance of the medium to the motion must be completely given by either a real or apparent viscosity factor. Changes therefore, in the nature of the environment are reflected in the motion of the particles.

Gel Structure

Imagine that we may have two types of gel. In one the structure is so fine grained, the discontinuities so small that the medium is effectively homogeneous. The formation of a gel would appear to be accompanied by an increase in the viscosity of the medium. The distribution of the Brownian motion displacements (of foreign particles) at various stages could be represented by a family of curves obtained by substituting various values for η in the equation for a normal distribution. If on the other hand, a gel formed in which the discontinuities were of the same order of magnitude as the displacements, the distribution of the displacements would obviously be abnormal, particularly by an elimination of the displacements equal to or larger than the discontinuities.

The influence of the nature of the environment may also be detected by changes in the other quantities mentioned. Thus it was seen that in a homogeneous medium, the value for the magnitude of the concentration fluctuations is determined solely by the average concentration (Equation 3 and 4). The formation of a gel of the homogeneous type would not therefore change the magnitude of the concentration fluctuations. In the presence of discontinuities, the fluctuations would be hindered and the value for the mean lowered. From point to point in the gel, one should expect different values for v and differing values of the mean fluctuation. An analogous effect in the case of various types of gels would show itself in the frequency of occurrence of various concentrations in a small element of volume. (Equation 2.)

The application of this method of gel study involves the introduction of neutral foreign particles the Brownian motion of which may be studied. Mercury particles prepared by electrical pulverization are useful for this purpose.

The film records the formation of a weak gelatin gel containing mercury particles. The gelatin was clarified so as to be practically optically empty under the ultramicroscope. A preliminary survey of such films indicates that in the first stages, the gelatin influenced the Brownian motion as if the viscosity were gradually increasing, although in the later stages, the motion appears to be localized more highly than would correspond to a simple viscosity effect. It is possible that in this stage, the mercury particles adhere to the gel material. In the case of some gels, as of manganese arsenate and dibenzoyl cystine, the

beginning stages of gel formation differ in that the gradually increasing apparent viscosity is not so evident. The formation of the gel takes place comparatively suddenly after what may be called a period of induction. This behavior may be due to the fact that the formation of a manganese arsenate or dibenzoyl cystine gel is preceded by a condensation from molecularly dispersed to colloidally dispersed state. This condensation is dependent upon a state of supersaturation and the appearance or formation of nuclei in sufficient number and size to allow the deposition of the material causing the supersaturation. The period of induction in gel formation probably corresponds to this initial supersaturation and condensation. These initial conditions are presumably lacking in the case of the formation of a gelatin gel.

The dibenzoyl cystine gel [5] is particularly interesting. In all cases in which a gel had certainly formed, long thin fibers entangled together made up the visible gel structure. The viscosity of the medium between the fibers appeared to be practically unchanged. The fibers themselves were also in motion. Short ones twist and turn about like flexible rods. Longer ones fixed at one or both ends wave like cords in a breeze. Although I have not succeeded in preparing dibenzoyl cystine gels which do not show the presence of the fibers in the ultramicroscope, it is not safe to conclude that they form the essential gel structure. It may be recalled that McBain [6] observed as a rule, the presence of fibers in soap gels, which presumably were curd fibers. In fresh gels, he reports, the fibers may be very few.

The film record of the melting of a gelatin gel containing mercury particles shows some interesting features. The molten gelatin was placed in the quartz cell and allowed to set. One might suppose it to be uniform. That it actually is not is shown by the deportment of the mercury particles during the melting stages. The resistance to the Brownian motion decreases non-uniformly. At some points, the gel structure may suddenly break down locally. Channels sometimes appear through which the broken gel structure and the suspended mercury particles flow.

Formation of Colloid Particles

The effectiveness of the kinoultramicroscope is shown particularly well in connection with the study of processes which take place with greater rapidity than gel formation. Thus an alkaline solution of bismuth tartrate is reduced by light to give a colloid solution of bismuth. The kinetics of the process may be followed in the ultramicroscope with special advantage. The cell in which the reduction takes place is so thin that the absorption of light may be neglected. The entire solution

[5] This gel has been studied by Gortner and Hoffmann, *J. Am. Chem. Soc.*, 43, 2199 (1921), and by Wolf and Rideal, *Biochem. J.*, 16, 548 (1922).
[6] *Loc. cit.*

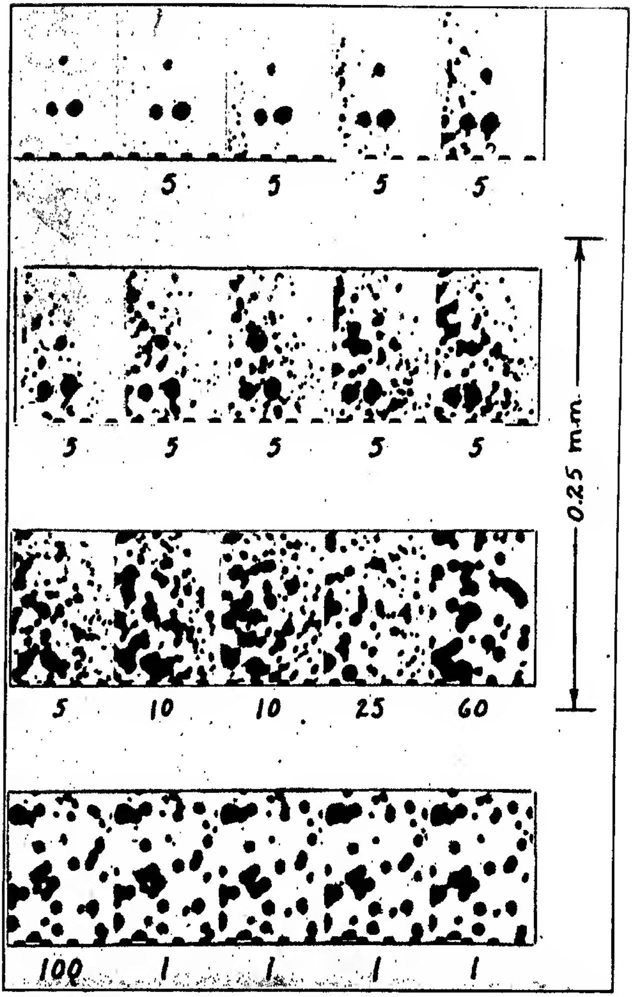

FIG. 4.—Formation of a bismuth sol. The number beneath each picture gives the time (in time units of 0.83 seconds) intervening between it and the preceding picture.

being studied may be considered as equally illuminated. The conditions approximate very closely those of volume condensation. This formation of a bismuth sol is one of a relatively small number of examples of phase formation which can be followed and studied directly from the point of view of the new particles formed. The factors involved in the formation of a new phase are fundamentally important

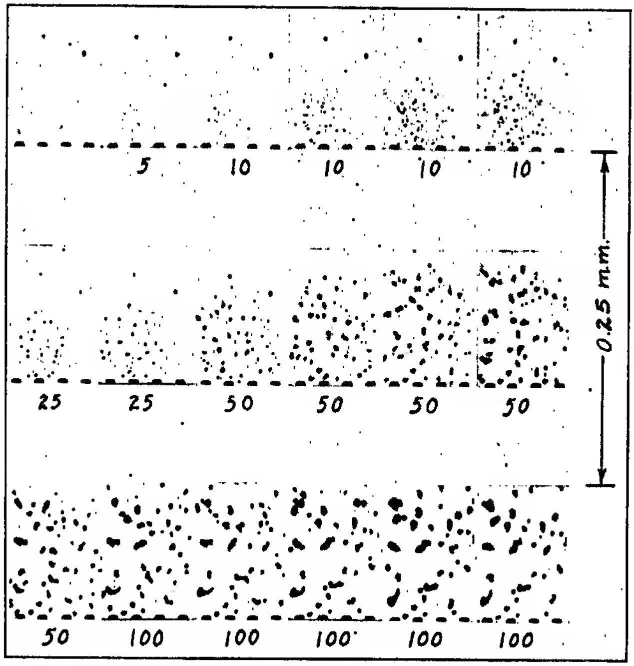

FIG. 5.—Stages in the coagulation of a silver sol. The numbers give the intervals between pictures in time units of 0.76 seconds.

in connection with the formation of disperse systems by condensation. The process illustrated in the film (see Figure 4) took place in approximately 3 minutes. In this case, the specimen was illuminated constantly. The reduction, however, is not due to the heating effect of the light, for no reduction takes place upon heating such a solution in the absence of light. It should be noted that on the basis of the intensity of the diffracted light, the particles are very unequal in size, contrary to what one might expect in the case of volume condensation.

Coagulation of Colloid Particles

The coagulation of a colloid solution may be recorded quite prettily with the kinoultramicroscope. Such a record was made of the photocoagulation of a Carey Lea silver sol (see Figure 5). The original sol was very highly dispersed in the form of amicrons. Under the

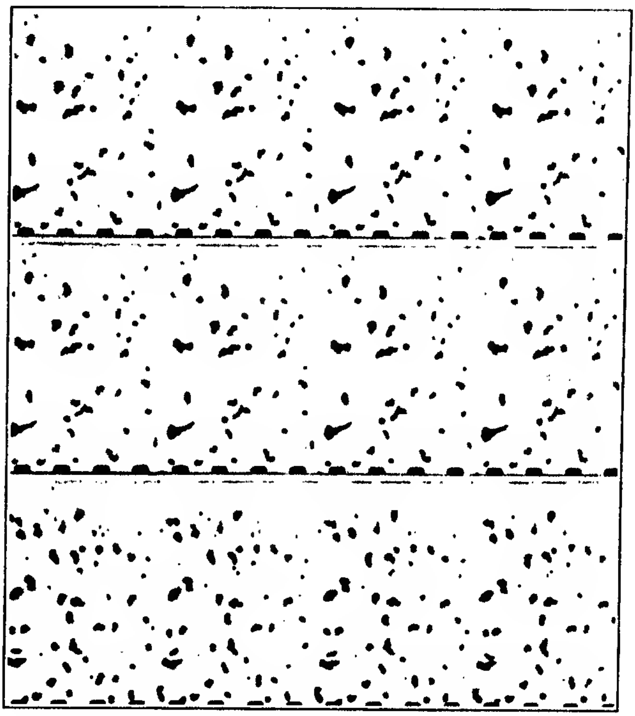

Fig. 6.—Agglomerates of considerable size execute a wriggling type of Brownian motion

intense illumination of the cardioid condenser, coagulation sets in in the course of a few seconds, leading to the formation of secondary particles large enough to be visible and to photograph. These continue to grow and agglomerate to form large masses. It is striking that even after the agglomerates have reached a considerable size, they continue to execute a wriggling type of Brownian motion, whereas rigid

particles of the same size would be motionless. This motion may be seen in Figure 6. The unions between the particles making up the agglomerates must be loose and flexible. In fact the constituent diffrac-

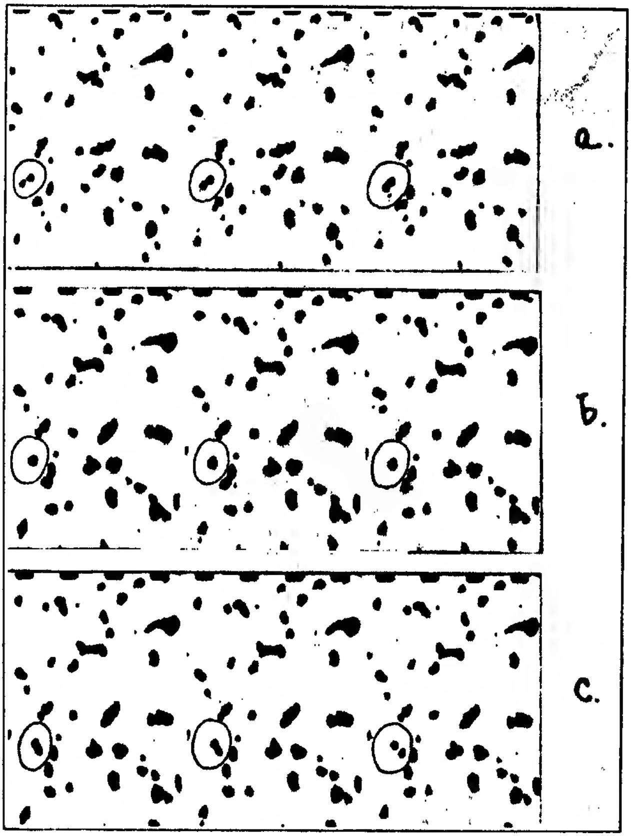

FIG. 7.—Reversibility of coagulation. (a) Stages during the union of two silver particles. (b) The two particles bound together for 13 seconds. (c) Separation of the two particles. The time interval between successive pictures of each group was 0.76 seconds.

tion rings forming the image of the agglomerate can actually be seen visually and in the photographs. Although the particles themselves are below the resolving power of the microscope, the distances between the particles are great enough to be easily resolved, as attested by the

individual diffraction rings. It is also possible, however, that the visible particles of an agglomerate are cemented together by invisible ones.

It is of considerable interest that the coagulation in the first stages is reversible spontaneously. Two particles may sometimes combine for a short time and then separate again. Figure 7 illustrates how two particles combined and moved as one for 13 seconds before separating. If this is a general phenomenon in "slow" coagulation in the sense of Smoluchowski's theory, it necessitates a modification of that theory. It is insufficient to evaluate the probability of encounters to form agglomerates; an additional term should be introduced taking account of the probable "life" or duration of a combination.

The examples discussed in this preliminary paper by no means exhaust the possible useful applications of the kinoultramicroscope. After the completion of the investigations in progress, it is hoped that the method of study may be found helpful in attacking other problems of colloid science.

Note

The cinematographic illustrations presented with this paper were:

The superposition of Brownian motion and gravity sedimentation of mercury particles.

The Brownian motion of sulfur particles without appreciable sedimentation.

Spontaneous concentration fluctuations in a gold sol.

Formation of a gelatin gel as indicated by the motion of mercury particles suspended in the gel.

Melting of gelatin gel, also indicated by the foreign particles.

Formation of bismuth sol by photoreduction of a bismuth solution.

Photocoagulation of a Carey Lea silver sol.

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A NEW METHOD FOR THE DETERMINATION OF THE DISTRIBUTION OF SIZE OF PARTICLES IN EMULSIONS

ALFRED J. STAMM

It has been pointed out by Svedberg[1] that the adequate characterization of a colloid solution requires a knowledge of the distribution of size of particles in the colloid system. Since the particles of a colloid system are usually not susceptible to direct microscopic measurement on account of their small size, the determination of the distribution of size of particles generally depends upon the application of the resistance law, $f = 6\pi\eta r v$ where f is the friction developed when a sphere of radius r moves with the uniform velocity v in a liquid possessing the viscosity η.[2]

Very few such studies have been made upon emulsions in which the disperse phase rises under the influence of gravity. The methods which have actually been used have involved either the direct measurement with the microscope of the sizes of a large number of drops of the disperse phase, or the application of the resistance law to observations, with the aid of the microscope, of the rate of rise of a statistically sufficient number of drops.[3] Both methods are quite laborious, and in the case of small drops, the accuracy of the first method may be rather low.

For certain classes of emulsions, the distribution of size of particles may be determined very simply, however, by observing the rate of change in the apparent density of the upper portion of an emulsion into which the disperse phase is rising. The observations, when properly interpreted, give the rate of accumulation of the disperse phase into the upper portion. From the time-accumulation curve so obtained, the distribution curve may be derived in the usual fashion.[4]

Consider a tube with a capillary sidearm as illustrated in Fig. 1, and imagine the vertical tube to contain an emulsion while the capillary tube contains the pure dispersion medium. With the passage of time, the disperse phase rises, passes the plane A and causes an equal volume of the dispersion medium to move across the plane A into the lower

[1] Svedberg and Estrup, *Kolloid-Z.*, 9, 259 (1911).
[2] Svedberg gives an excellent discussion of the determination of distribution of size of particles in his book, "Colloid Chemistry," A. C. S. Monograph (1924).
[3] Svedberg and Estrup, *loc. cit.*
[4] Odén, Bull. Geol. Inst. Upsala, 16 (1916). Svedberg, "Colloid Chemistry," p. 144 ff.

portion. The net result, however, is a decrease in the average density of the liquid above the plane A and a corresponding and compensating decrease in the height of the liquid in the capillary tube. Therefore the rate of change of the meniscus in the capillary tube is a measure of the rate of deposition of the disperse phase into the region above the plane A (which is thus equivalent to the balance pan in the downward sedimentation method of Odén).

In more exact language, let A and s be the cross-sectional areas of the

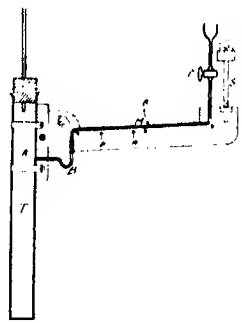

FIG. 1.

plane A and the capillary tube respectively; d_m and d_p the densities of the dispersion medium and the disperse phase respectively. As P grams of the disperse phase, rising, passes the plane, A, it displaces an equal volume of the dispersion medium, or $P\frac{d_m}{d_p}$ grams. Therefore the decrease in pressure per square centimeter on the plane A is

$$\frac{1}{A}\left(P\frac{d_m}{d_p}-P\right)=\frac{P}{A}\left(\frac{d_m-d_p}{d_p}\right).$$

To compensate this change, the meniscus falls in the capillary tube from a to b, changing the pressure by the quantity $d_m(a-b)sin\theta$. But the fall in level in the capillary tube introduces into the wider tube a quantity of liquid given by $d_m(a-b)s$ which raises the level and increases the pressure in the wide vertical tube by the quantity $d_m(a-b)\frac{s}{A}$. Equating the total changes in pressure in the vertical and capillary tubes gives

$$-\frac{P}{A}\left(\frac{d_p\cdot d_m}{d_p}\right)+d_m(a-b)\frac{s}{A}=-d_m(a-b)sin\theta$$

and solving for P finally yields

$$P = \frac{d_p \cdot d_m}{d_m - d_p} [A \sin\theta + s][a - b].$$

The value of P obtained when a and b are the original and final positions of the capillary liquid should agree with the quantity of disperse phase in the volume below the plane A as determined by analytical means, if no "creaming" has taken place before the original observation a, and if the separation of the disperse phase from the region below A is complete. An accumulation curve may then be constructed to show the relationship between P and time.[5]

Apparatus

The apparatus (see Fig. 1) was mounted on a wooden frame and clamped securely to a vertical rod in a thermostat. A spirit level L was fastened to the wooden frame so as to be level when a plumb line hung parallel to the side of the tube T. A leveling screw S fastened to a cross bar in the thermostat served to adjust the level of the apparatus. The bend B in the capillary tube prevented the disperse phase from passing up through the capillary. A glass scale was mounted under the capillary tube, and readings made with a microscope. The tube T was provided with a stopper through which a glass tube drawn out to a short orifice extended in order to cut down the evaporation of the volatile disperse phase.

The angle of the capillary was determined by two different methods. The first of these was to measure the length of two projected sides of the right triangle formed between the tube T and plumb line when the apparatus was clamped with M horizontal. This gave 0.0765 as the average value of sin θ. In the other method, the tube was clamped in p'ace in the thermostat with T vertical. The apparatus was filled v ı water so that the meniscus in M stood near the lower end. A vertical micrometer screw was mounted above the tube T with a glass thread fastened to its lower end. This was brought just into contact with the water surface, and readings taken on the micrometer screw and the capillary scales. The micrometer screw could be read to 0.001 cm., and check readings made to 0.002 cm. The capillary scale was read to 0.005 cm. with maximum variation of 0.01 cm. From the change in height of the liquid in the tube T upon addition of successive portions of water, and the corresponding capillary readings, the angles for different portions of the capillary were determined. These differed by a maximum of 3% for different positions along the scale, and gave an average value for sin θ of 0.0767. The appropriate value was used for the sin θ at different positions along the capillary.

[5] The principle of the method is similar to the sedimentation method of Wiegner (*Landw. Versuchsstat.*, 91, 41 (1919)), and of Ostwald and Hahn (*Kolloid Z*, 30, 62 (1922)), but so modified and improved that emulsions with a rising disperse phase may be studied.

The cross section of the tube T was determined just above and just below the manometer tube junction by means of the change in the vertical micrometer screw readings upon the addition of 5 cc. of water. The cross section was found to be 4.400 cm.[2] The height to the junction was 29.2 cm., and that of the liquid used was 39.2-39.6 cm.

The rate of evaporation was determined with a layer of benzene on the surface of the water in the tube T, and the drift down the manometer tube with time measured. This was done over a period of 36 hours, and an average movement of 0.009-0.01 cm. per hour noted. This correction, which was practically constant from hour to hour, was applied to the following observations. It has been possible to practically eliminate the evaporation drift by providing the vertical tube near the top with a supplementary side bulb also immersed in the thermostat. In this bulb is placed some benzene so that the region above the emulsion being studied is saturated with benzene vapor.

For studying water in oil emulsions (in which the disperse phase settles downward) it is expedient to place the juncture of the capillary side tube nearer the bottom of the vertical tube. For such emulsions, this method of size determination is particularly advantageous, since the usual method of microscopic measurement is quite unsatisfactory on account of the volatility of the dispersion medium.

Preparation of Emulsions

The studies reported in this paper were made with benzene in water emulsions. The benzene was purified according to the usual method for removing thiophene. The fraction used distilled at 78.55-78.60° (739.6 mm.). The density at 25° compared with water at the same temperature was 0.8754. The soaps were prepared from pure fatty acids according to the method of White and Marden.[6]

The emulsions were prepared under the following arbitrary conditions. One cc. of the emulsifying agent dissolved in either benzene or water was placed in each of two 120 cc. oil sample bottles. The required amount of benzene was then added, and enough water to make the volume up to 100 cc. The water was added in 20 cc. portions, and the mixture was shaken for 15 seconds between additions. The samples were then shaken by hand for 5 minutes at an approximate rate of 100 shakes per minute. When desired, the emulsions were further emulsified by means of a Briggs' homogenizer.[7] The homogenizer was used with a constant pressure difference of 60 cm. of mercury. The pressure over the emulsion in the receiver was either approximately 14 or 74 cm. of mercury.

[6] White and Marden, *J. Phys. Chem.*, **24**, 618 (1920).
[7] Briggs, *J. Phys. Chem.*, **19**, 223 (1915).

Determination of Size Distribution

In accordance with the theoretical considerations already given, accumulation curves were constructed with P as ordinates and the corresponding time in hours as abscisssæ. Fig. 2 is a typical accumulation curve. All of the accumulation curves were found to be quite smooth and very similar in general appearance.

The distribution curves were determined from the accumulation curves according to the previously cited method given by Svedberg, by taking dS as the distance between the ordinate intercepts of two suc-

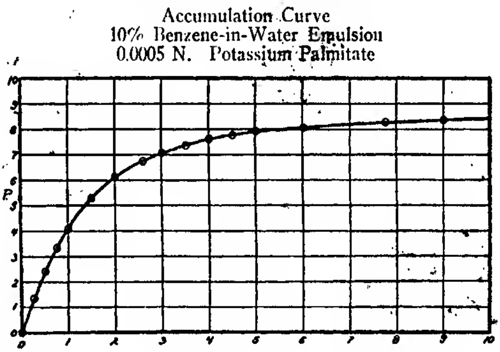

Fig. 2.—Time in hours.

cessive tangents to the accumulation curve. The radius of the particles in μ (10^{-4} cm.) is plotted against the ratio of the change in weights dS to the change of radius dr. Hence the area under any portion of the curve represents the actual weight of benzene dispersed in particles whose sizes vary between those values represented by the bounding abscissæ. The greatest weight of the benzene in the emulsion was dispersed into particles the size of which was given by the maximum in the distribution curve. Curve 2 of Fig. 3 is the distribution curve derived from the accumulation curve given in Fig. 2.

The degree of reproducibility of the distribution curves is also shown in Fig. 3. The 3 curves are for 3 different emulsions with potassium palmitate as stabilizer prepared under as nearly identical conditions as possible, being homogenized twice with the pressure in the receiver approximating 14 cm. mercury. The 3 curves are similar in type, and the maximum appears at very nearly the same radius for all 3 curves. The values for the maxima are

Curve	1,	maximum at	8.8 μ
"	2,	" "	9.0
"	3,	" "	8.6

The actual values of the masses do not check so well due to the experimental difficulties in emulsifying every bit of the material. A little of the benzene invariably sedimented out before the initial reading could be taken. This usually varied from 0.4-1.0 grams as estimated by the depth of the surface layer of benzene in the vertical tube. This estimate was of course a minimum value, as a considerable portion of the emulsion particles that had risen above A still remained dispersed. For example, in the case of Curve 1, 174 cc. of 10% benzene emulsion was used, *i.e.*, 17.4 cc. of benzene. Just after the first reading, 1.75 cc.

Distribution Curves.
10% Benzene-in-Water Emulsions
0.0005 N. Potassium Palmitate

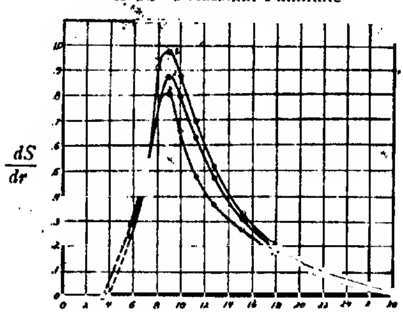

FIG. 3.—Radius of particles in μ.

of benzene had settled out. Subtracting this, 15.65 cc. of benzene remained dispersed, and $15.65 \times \frac{29.2}{39.3} = 11.6$ cc. (or 10.15 grams) had yet to cross the boundary A. The experimentally determined amount settled out up to the time when the motion of the capillary liquid became that of the evaporation drift (17 hours) was 9.15 grams. This shows that some very finely dispersed benzene had not settled out, or more probably, that the initial estimate of the amount settled out just up to the time of the first reading was low.

The final point giving the minimum size was not determined in the same way as the other points. It was obtained by considering that the sedimentation was completed at the time when the movement of the capillary liquid became that due to evaporation. Of course, such was not really the case, and the last section of the curve is therefore merely dotted. In reality, the curve should probably become tangent to the radius axis more gradually than the dotted line indicates. Superficially, the curve would resemble a Maxwellian distribution curve.

In Figs. 4 and 5 are given curves showing that the position of the maximum is not greatly changed by considerable changes in the concentration of the emulsion. The maxima occur for the following radii—

Curve 1,	maximum at	8.9 μ
" 2,	" "	9.0
" 3,	" "	10.2

The 3 emulsions were prepared using the same procedure, and each was homogenized twice under low pressure (14 cm.). A comparison of the

Effect of Change of Benzene Concentration on Distribution of Sizes
0.0005 N. Potassium Palmitate
1. 5% Benzene *2.* 10% Benzene *3.* 15% Benzene

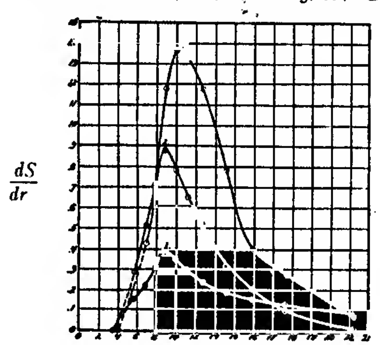

FIG. 4.—Radius of particles in μ.

curves shows that the efficiency of emulsification is but slightly changed by changes in the concentration of the emulsion, even when the total quantity of emulsifying agent remains constant. Furthermore, the rate of settling is not greatly changed due to the mutual interference of the settling particles. The apparent increase in the average size of particle with concentration may be real, and due to the fact that the concentrated emulsions contained relatively less emulsifying agent; or the drops may actually have been of the same sizes, but settled faster in accordance with the theoretical calculations of Cunningham [8] upon the rate of fall of a cloud of uniform particles.

Fig. 5 gives results for emulsions of benzene in water stabilized with potassium oleate. The method of preparation was the same as used in obtaining the results just discussed.

Curve 1,	maximum at	10.4 μ
" 2,	" "	11.8

Comparing Curves 1 and 2 with the corresponding curves of Fig. 4

[8] Cunningham, *Pro. Roy. Soc., London*, 83, 357 (1910).

(Curves 2 and 3), one may note that the potassium oleate gives a maximum corresponding to a larger size of dispersed particles. According to the theory of Langmuir [9] and Harkins [10] as partially verified experimentally by Finkle, Draper and Hildebrand,[11] the oleate should give the larger particles as the cross sectional area of a hydrocarbon group containing a double bond is greater than that of a saturated hydrocarbon group.[12] This presumably tends to give less curvature to the monomolecular layer of oriented molecules, on the basis of the "wedge" theory of emulsions.

Effect of Change of Benzene Concentration on Distribution of Sizes
0.0005 N. Potassium Oleate
1. 10% Benzene *2.* 15% Benzene

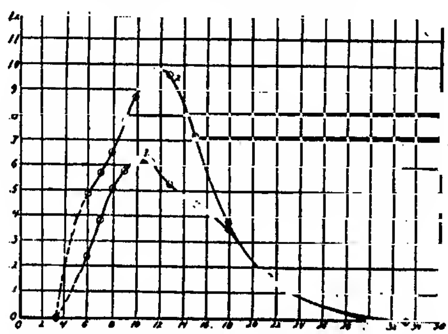

FIG. 5.—Radius of particles in μ.

The influence of homogenization is shown by the curves of Fig. 6. The results may be summarized thus,

Curve	1,	maximum	at 12.0 μ	(no homogenization)
"	2,	"	" 8.9	(homogenized 2 times at low pressure)
"	3,	"	" 7.0	(" 4 " " " ")
"	4,	"	" 21.3	(" 2 " " high ")

Homogenization with a low pressure over the emulsion in the receiver (14 cm. mercury) obviously increases the degree of dispersion of the benzene in water emulsion stabilized with soap. However, with gelatin as stabilizer, a benzene in water emulsion is broken completely by a single homogenization. Emulsions stabilized with free fatty acids, as palmitic acid or capric acid were found to be partially broken by such treatment. With atmospheric pressure in the receiver, homogenization

[9] Langmuir, *Chem. Met. Eng.*, **15**, 468 (1916).
[10] Harkins, *J. Am. Chem. Soc.*, **39**, 541 (1917).
[11] First National Colloid Symposium Monograph, June (1923). *J. Am. Chem. Soc.*, **45**, 2780 (1923).
[12] Langmuir, *J. Am. Chem. Soc.*, **39**, 1868 (1917). N. K. Adam, *Proc. Roy. Soc., London*, **99**, 336 (1921); **101**, 452 (1922).

tended to break all the emulsions studied. The surprising difference in the effects of the two methods of homogenization may be due to the difference in the rate of evaporation of the benzene in the two cases. Thus, homogenization with low pressure allowed a rapid evaporation of the benzene, and caused thereby a pronounced foaming. The formation and breaking of bubbles and laminæ is probably quite effective in increasing the dispersity of the emulsion.

Weight Distribution Curves for benzene in water emulsions with po-

Effect of Homogenization of 5% Benzene-in-Water Emulsions
0.0005 N. Potassium Palmitate.

1. No Homogenization
2. Homogenized 2 Times (Low Pressure)
3. Homogenized 4 Times (Low Pressure)
4. Homogenized 2 Times (High Pressure)

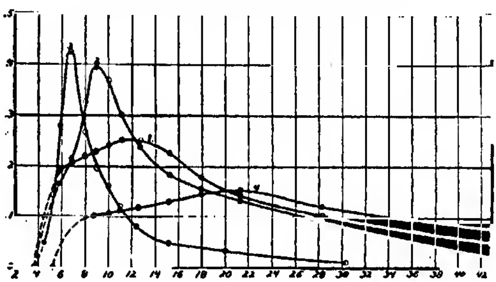

FIG. 6.—Radius of particles in μ.

tassium palmitate as stabilizer have been determined by microscopic measurement of 1500 particles for each determination. These curves checked satisfactorily with those obtained by the sedimentation method herein described.

Further experimental data and a detailed discussion of the bearing of the investigation upon theories of emulsification will furnish the material for a future paper.

The author is indebted to Elmer O. Kraemer for suggesting the appropriate modification of the Wiegner sedimentation tube and for helpful advice during the course of the work.

SUMMARY

1. A new and simple method for determining the distribution of size of particles in emulsions with a rising disperse phase has been described.

2. It has been shown that distribution curves could be checked with

a fair degree of accuracy for two emulsions prepared under the same conditions.

3. The position of the maxima in the distribution curves was found to be but slightly changed by changes in the concentration of the emulsions.

4. As a stabilizing agent, potassium oleate tends to give an emulsion with larger drops than does potassium palmitate, in accordance with the wedge theory of emulsification.

5. Homogenization under low pressure in the receiver was found to increase the dispersity of the benzene particles stabilized with potassium soaps, but tended to break the emulsions containing gelatin or free fatty acids as stabilizers. Homogenization with atmospheric pressure in the receiver caused a breaking of the emulsions studied.

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THE PROPERTIES OF CLAYS

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Clays are soft or consolidated rocks consisting essentially of hydrous silicates of alumina of the type $Al_2O_3 2SiO_2 2H_2O$, which, when admixed with water, are capable of being molded under a *low* pressure and which retain the shape imparted upon removal of the pressure. Furthermore, the clays harden upon the application of heat and finally form a rocklike mass of varying degrees of porosity. Emphasis must be put on the qualification that wet clay can be molded under a low pressure, corresponding to the pressure of the hand, since many other materials can be shaped under high pressures.

The chemical and physical characteristics of the clays vary with the geological processes which have produced them. We may thus distinguish at once the primary clays, which are found upon the original site of their decomposition from feldspathic rocks, from the secondary type which includes the vast quantity of clays transported from the place of formation and deposited in flowing or quiescent water. The possible variations which tend to degrade the pure primary clays or kaolins to other types such as the secondary or plastic kaolins, the so-called ball clays, the fire clays, shales, the alluvial and glacial clays are infinite. In like diverse fashion is it possible that impure clays may become purified and again transformed into materials of a higher type. It is impossible to discuss here the many geological processes which are involved in the making of clays.

While in the primary kaolins we find ample evidence of the presence of crystalline clay the anisotropic character of the substance is almost entirely destroyed in the secondary clays and the state of aggregation changes from a loose to a more compacted structure with geologic age. The gross and the minute structure of the clays are of extreme importance in their industrial application, in fact, they determine in a very large measure the use to which they can be put. This point may be illustrated by referring to the different periods of Chinese porcelain. The several types that have been produced have changed from one to the other with the change in the raw materials and the interruptions in porcelain manufacture have been due perhaps to the exhaustion of known deposits.

The properties of the clays may be considered under the following headings:

1. Aggregate structure.
2. Mineral composition and micro-structure.
3. Fineness of grain.
4. Chemical composition.
5. Properties of clay suspensions.
6. Properties in the plastic state.
7. Properties in the dry state.
8. The effect of heat.
9. Properties in the fired state.
10. The testing of clays.

Aggregate structure. The gross structure of the clays, whether it is loose, compacted without bedding planes or laminated, and the degree of compactness is a vital factor in the behavior of clays and must never be disregarded. Obviously, these conditions affect the degree of agglomeration of the ultimate clay particles and their cohesion. Certain industrial applications depend upon the looseness of the structure while others demand compacted materials. The effect of the geological history of the clays persists throughout all of the stages through which they pass in the course of fabrication and cannot be eradicated except by the most intensive and costly treatment. Thus, there is a vast difference between the loose, soft structure of kaolin and the compactness of flint clay which are identical in chemical composition. The same thing is true of the halloysites, indianite and other hydrous silicates of alumina. An alluvial red burning clay shows striking differences from a red shale although here again the chemical composition may be practically the same.

In a practical way the loose clays are easily differentiated from the compacted materials by noting the time of slaking in water of specimens previously dried at 110° C.

Mineral composition. Microscopic examination of clays may reveal the presence of crystalline kaolinite or its entire absence. In the latter and more frequent case we find cloudy, flocculent aggregates of clay matter and with it, grains of quartz, sericite, feldspar, muscovite, pyrite, calcite, rutile, siderite, gypsum and many other minerals. Quartz is the most commonly associated mineral, followed by muscovite and feldspar. Organic matter in many modifications, from humus to graphite, is usually present.

Many attempts have been made to classify clays through their complete solubility in sulphuric acid and their partial solubility in hydrochloric acid. The claim has been made that the halloysites are entirely soluble in HCl, while the clay substance akin to kaolinite requires to be unlocked by fuming H_2SO_4. The so-called mineral analysis of clays depends upon the solubility of the clay matter in the last named reagent,

leaving the granular quartz, feldspar and mica undissolved. But the method is far from being an accurate one, and the ultimate analysis is now preferred. From the analytical results the percentages of clay matter and of the principal accessory constituents are computed under the assumption that the composition of the clay substance is: Silica 46.52 per cent, alumina 39.53 and chemically combined water, 13.95.

The microscopic examination of the clay substance is usually disappointing, owing to its colloidal character and the best we can do is to estimate the amounts of quartz, feldspar, mica and other crystalline constituents. From the work of Bragg it appears that even the fine clay substance possesses crystalline structure as indicated by means of x-ray analysis.

Many conjectures have been made as to the chemical structure of the kaolinite molecule, based on the assumption of a series of theoretical silicic acids, or the possible existence of alumino-silicic acids (kaolinic acids). W. and D. Asch [1] have even proposed that we are dealing here with alumino-silicic acids which are ring compounds, analogous to benzene and its derivatives.

As a matter of fact our knowledge of the structure of kaolinite consists only of conjectures and remains to be clarified through the aid of the x-ray spectrum.

Fineness of grain. Much attention has been given to the subject of the fineness of grain of clays through the use of mechanical separation employing the principles of sedimentation and elutriation as elaborated by soil chemists. In the former the average grain sizes are computed from Stokes' law and in the latter by various empirical relations between the speed of the current of water and the size of grain. The Schoene elutriator is frequently employed for the work of classifying clays according to the scheme of Seger proposed many years ago who adopted the following gradation of sizes:

Speed of water current	Size in mm.	Designation
0.18 mm. per second	0.010–0.005	Clay substance
0.70 mm. per second	0.025–0.010	Silt
1.50 mm. per second	0.040–0.025	Dust sand

Recent estimations of the fineness of kaolins and plastic clays by Stark [2] by a simple sedimentation method, in which the specimen of clay, thoroughly worked up with water, is introduced over a 3 per cent sugar solution give an approximation of the grain sizes with which we are dealing. In this work the grain sizes are computed from Stokes' formula:

$$r = \sqrt{\frac{9kc}{2(S_k - S_f)}}$$

where r = radius of particles
k = viscosity of liquid
c = velocity of sedimentation
S_k = specific gravity of clay
S_l = specific gravity of liquid.

By means of fractional sedimentation it was found that Zettlitz kaolin had particles of a mean diameter of 4.8 and a plastic bond clay of 3.6 microns. The former also contained 32 per cent of coarser particles, 11-49 microns; the latter, 21 per cent of particles ranging from 9-40 microns. Both clays consisted of a portion of material much finer than the mean diameter, thus bringing these clays within the field of suspensoids. The number of particles per gram of Zettlitz clay was approximately 6.6×10^9 and for the plastic clay, 15.8×10^9. The specific surface of the former in sq. cm. was 4810 and of the latter, 6410.

The exact determination of the mean size of grain of clays is quite difficult by either method of separation owing to the variations introduced through the temperature fluctuations of the liquid, the effect of electrolytes, the static effect of the containers, etc.

The fineness of clays is often expressed by means of a surface factor:

$$S = p_1 1/d_1 + p_2 1/d_2 + p_3 1/d_3 \text{ etc.}$$

in which S = surface factor, $p_1 p_2 p_3$ = percentages of the different fractions and $d_1 d_2 d_3$ = mean diameter of each fraction. Ultramicroscopic examinations have verified the existence of a wide range of particles from the most minute to those which barely show Brownian movement.

Chemical composition. The composition of clays varies widely from nearly pure $Al_2O_3 2SiO_2 2H_2O$ to those materials, like brick clays, which contain large quantities of quartz, ferric oxide, calcium carbonate and mineral débris of all kinds. There is hardly a rock which shows such enormous fluctuations in composition and which, by the way, is so difficult to sample. It is obvious that we must expect to find equally large variations with respect to the physical properties of the materials. This may be illustrated by the fact that pure kaolin fuses at 1750° C. while some of the impure clays melt as low as 1100°. The most effective mineral impurities which bring about the most radical changes are the feldspar, the oxides of iron and calcium carbonate. With the presence of the iron compounds the color of the clay is altered from white to red and black, when fired.

Clay suspensions. In preparing a suspension of a soft clay in water it is evident that the coarser particles will settle out rapidly, while the clay matter proper will remain suspended for a considerable period.

A cloudy suspension may persist for weeks. Suspensions of this kind are exceedingly sensitive to the presence of electrolytes, being deflocculated by alkaline and coagulated by acid reagents and salts. We are hence dealing here with coarse suspensions since the particles are larger than 0.1 micron. The behavior of clay-water systems is strictly analogous to typical colloidal suspensions corresponding to this size of particles. This statement is supported by other facts, such as the observed Brownian movement of the particles, the formation of the Tyndall cone upon passing a beam of polarized light through the suspension, the marked adsorption of inorganic and organic ions, the sharp deflocculation and coagulation phenomena referred to above and the travel of the particles in an electrical field towards the anode. It appears thus that the clay particles correspond to an electronegative colloid.

But it must be kept in mind that we are dealing here with a wide range of particles, varying from coarse grains to those of ultramicroscopic fineness.

Schloesing [3] was first to attempt the separation of colloidal material from clay and succeeded in removing from a kaolin 1.47 per cent of a superfine substance, which remained in suspension for 27 days. Mellor [4] removed 0.05 per cent of glue-like material from a ball clay. Undoubtedly, the amount of substance approximating the state of a colloidal solution is very small but that classifiable as a coarse suspension is considerable. Up to the present time no accurate classification of the dispersed phase of clays has been made. The best means of attack would seem to lie in the combination of gravimetric and optical methods, or in the employment of cataphoresis.

The study of the water-clay systems has followed closely the progress of knowledge of colloid chemistry in general and has given rise to similar controversies. But in the study of clays we must always keep in mind that we are dealing here with more complex and impure systems, far different from the purified sols and gels of the laboratory. Considering the subject from the general standpoint of the hydrogen ion concentration we should expect the pH value to be the governing factor as to the stability of a clay suspension. This appears to be true for soils as well as clays.

As a class the clays react acid and their pH values, according to Hall,[5] range between 3.10 and 7.27, rejecting in his results those materials which are obviously high in soluble salts. These figures refer to the water extract from the clays, the ratio of water to clay being 4: 1. Hall observed also that washing reduced the acidity or alkalinity of the clays but very slowly. Aging of the clays likewise seems to have no marked effect upon the pH value. According to the character of the clay the iso-electric point lies between pH 2.7-4.0. The point of maximum deflocculation varies from pH 11-12 and was found by Hall

to be less sharp than that determined by Arrhenius.[6] The study of the deflocculation of clay by means of industrial viscosimeters bears out the findings of Hall.

Speaking of clay-suspensions in a general way we may say that they are acid in character and the particles are charged negatively. The addition of lithium, sodium and potassium, effective in the order named, increases the negative charge until at a definite point further charges of alkali decrease the charge on the particles. The point of maximum charge represents maximum deflocculation. On the other hand, upon the addition of acid the particles become positively charged and coagulation and settling occurs. The maximum rate of settling takes place at the iso-electric point. This explanation of these phenomena by Mattson[7] agrees with the work of previous investigators.

In the coagulation of clays the valence of the adsorbed ion is an important factor and hence the presence of salts, such as the sulfates of lime and aluminum, are very detrimental in the deflocculation processes applied in the industry. For this reason many clays must be ruled out from such uses.

The powerful adsorbing power of clays is well known, and it is found to take up the basic ion, there being invariably found an excess of the acid ion in solution. Thus Bencke[8] found that 1 gram of kaolin adsorbed 0.0038-0.0169 gram of copper oxide from a 4 per cent solution of copper sulfate. Clays take up dyes, according to Arrhenius, in proportion to their molecular weights. As a rule the adsorption takes place according to the general adsorption equation but the value of the latter as a criterion to distinguish between adsorption and chemical combination may be questioned. In the case of the alkali hydroxides it is certain that chemical combination occurs even at low concentrations.

In practice, the fluidity of clay suspensions is used to determine the points of deflocculation and coagulation. For this purpose the simple flow tube viscosimeters are employed which answer the purpose satisfactorily and the curves correlating the amount of alkaline electrolyte with the fluidity for a given clay-water ratio yield an excellent graph of the progress of deflocculation. Torsion viscosimeters of the Coulomb or Couette types are hardly as satisfactory for industrial purposes. While we must agree that we are not dealing with true viscosity measurements the fact remains that very useful results are obtained rapidly with the flow tube devices.

As has been mentioned before the colloidal phenomena applying to clay are interpreted by ceramists either from the physical or the chemical standpoint. The former, represented by Purdy, considers them to be coarse suspensions and nothing more, without the entrance of chemical reactions. On the other hand, Ashley[9] accepted the view that deflocculation and coagulation are the result of chemical reactions. He says that the fine crystalline grains are enveloped by a colloid coating which

enters into "chemical reactions remarkably similar to those of the fats and soaps, in that the alkali sols of clay colloids are soluble and the acid gels of the clays are insoluble. Both soaps and clay sols may be salted out of solution by dissolving in the solution chemical salts that have more affinity for water than have the soaps or gels so salted out."

For all practical purposes Hardy's rule [10] suffices to explain the phenomena with which we deal in ceramics in that the precipitating radical is of opposite electrical sign to the colloid and that its effectiveness increases rapidly with the valence of the active ion. Coagulation is always the result of a decrease in the electrical charge and deflocculation of an increase. In all these cases we recognize for clays a definite minimum value which must be exceeded to bring about coagulation. Below this value the monovalent alkali cations cause an adsorption of OH ions and an increase in the charge, and the deflocculation caused by the repulsion of the particles reaches a maximum, below the minimum value of the sodium or potassium ions. Upon exceeding the minimum value, coagulation takes place. Protective colloids oppose coagulation and permit of the use of higher alkali concentrations. We may thus have alternating phases of deflocculation and coagulation until finally, with higher concentrations of the reagent these disappear.

Another strictly practical view which illustrates the fact that coagulated clays require a higher water content than deflocculated ones may be written as follows: Gel $x\ H_2O + (1 - x)$ free water $\rightleftarrows$ Sol $\frac{x}{n}$ $H_2O + (1 - \frac{x}{n})$ free water. Here $n > 1 =$ constant for a given clay. The illustration brings out the fact that there is an increase in free water when the material is deflocculated though it assumes that a portion of the water is held in some fixed manner by the clay particles in either state.

From the industrial standpoint clay suspensions are of interest with reference to the washing and purification of clays and the so-called casting process. The crude kaolins must be washed free from the quartz, feldspar and mica detritus, a process which is, in part, repeated during the preparation of semi-vitreous and vitreous porcelain bodies. In the former case the clay ore is stirred up with water and the thin suspension freed from the coarse material by sedimentation and sieving. Finally, the purified clay is separated from the water by decantation and filter pressing. It has been found that the addition of small amounts of NaOH, corresponding to the point of maximum deflocculation lowers the viscosity of the system sufficiently to bring about the more rapid and complete settling out of the coarser solids. This process, first suggested by Keppeler,[11] has been utilized in several instances.

For a time it was supposed that the electrical process of Count Schwerin,[12] in which the clay is removed from the suspension, by being collected on a positively charged, revolving cylinder was also instrumental in bringing about further purification but we know now that the more complete separation of the impurities was brought about by the preliminary treatment with NaOH. However this application of kataphoresis still has certain interesting possibilities.

In connection with the purification of clays for paper making it is sometimes necessary to add a trace of some blue colored substance to neutralize the slightly yellowish color of the kaolin. It has been found that with some clays the color neutralization may be brought about automatically by the addition of minute quantities of potassium ferrocyanide. The more colloidal ferric oxide there is present the more Prussian blue will be formed.

In the preparation of porcelain bodies the clays, admixed with the necessary quantities of ground quartz and feldspar are either stirred up with water, passed through fine silk lawns and filter pressed or they may first be ground in ball mills. This grinding increases the resulting plasticity of the clays. The loss in plasticity which might be suffered during the wet grinding due to the leaching out of alkali from the feldspar can be neutralized by the addition of aluminum chloride. In filter pressing care must be taken to work with as low a pressure as possible since high pressures decrease the plasticity of the clay body.

The casting process consists in the preparation of a heavy suspension of clay and the necessary non-plastics, usually of gravity 1.8, which is poured into plaster of paris molds. The latter, being porous, absorb water from the suspension (called slip) and cause the formation of a solidified layer of plastic material. According to the thickness of wall desired the slip is left in the molds for a shorter or longer time and finally the excess is poured out.

It is necessary that the amount of water left in the clay body be as small as possible in order that the shrinkage, which is proportional to the amount of water, be kept low. For the casting of heavier articles it would be hopeless to attempt the use of clay mixtures when merely suspended in water, since the amount of the latter required would be entirely too great. It has been found that by the introduction of alkali electrolytes, sodium carbonate and sodium silicate, it is possible to reduce the water content to a value hardly larger than that required for the material in the plastic state. At the same time the specific gravity of the slip can be carried as high as 1.9, which is of great advantage in this work, since it prevents the settling out of the non-plastics and yields dense, solid casts. In the successful operation of the process it is necessary to add just sufficient soda ash and silicate of soda

to bring about deflocculation. Small amounts of gallo-tannic acid increase the stability of the deflocculated suspension.

Many clays carrying appreciable amounts of soluble salts, such as the sulfates of calcium, magnesium and the alkalies can be deflocculated only difficultly, if at all. Sometimes the addition of $Ba(OH)_2$, which must be added before the alkali reagents are introduced, makes possible the use of such materials by fixing the sulfuric acid radical as $BaSO_4$. Clays carrying organic colloids are as a rule deflocculated much more easily than those free from such substances.

In practice the completed deflocculation is detected by the greatly increased fluidity of the slip and may be determined quite accurately from the amperage reading of the motor driving the mixing machine. Control of the proper condition of the suspension is maintained by means of density and fluidity determinations, using a simple type of flow tube viscosimeter.

Properties of clay in the plastic state. With the decrease in the water content the clay particles approach each other more and more until finally they are separated only by a film of water. With a given, fairly constant volume of water per unit volume of clay particles the degree of fluidity which characterizes the suspensions has disappeared and the system is said to be plastic. It is evident that there can be no sudden transition from the more to the less fluid state but that the change is a gradual one. In practice we have learnt to judge the degree of plasticity with considerable certainty and it is surprising what slight changes in this property may be detected by means of the potter's wheel. But as yet we do not know how to express plasticity in terms of physical constants.

Many attempts have been made to measure this property, most of which approach the subject by indirect methods. Thus Bischof proposed to measure plasticity by determining the length of a plastic clay column extruded from a die; Zschokke determined the tensile strength and extensibility of a plastic clay pencil and multiplied the two values; Jochum measured the angle through which a plastic clay bar, placed on a thin steel plate, can be bent before fracture occurs; the amount of water required to make a clay plastic or the per cent drying shrinkage in terms of the original volume have been suggested as criteria of plasticity. Again, the ratio of the volume of pore water to volume of shrinkage water has been suggested. Several workers have proposed as a criterion the amount of water held by clay suspensions of a definite fluidity and hence have brought in the so-called viscosity of such systems. Ashley [13] suggested a plasticity factor expressed by: $c\,s/f$, where c = relative colloid content, s = drying shrinkage and f = Jackson-Purdy surface factor as computed from the mechanical analysis of the clay. The colloid content is determined by the amount of malachite green ad-

sorbed from a 0.3 per cent water solution of the dye, expressed in terms of the quantity adsorbed by the same weight of a standard plastic clay. The transverse strength of clay bars dried at 110°, or their tensile strength, have been used as a measure of plasticity, or a composite factor, involving the use of this value combined with the drying shrinkage of the material. Stringer and Emery suggest as a measure the weight required to compress a 2 cm. clay sphere through a definite distance, the descent of the piston being limited by the formation of cracks.

Most of these methods have obvious defects but a number of them serve a useful purpose.

Recent direct attacks upon the problem are giving more hope of ultimate success, particularly the well known work of Bingham.[14] He establishes the proposition that plasticity is a complex property and involves at least two factors, a definite shearing stress, the yield value, and the mobility, as determined by measuring the volume flow of clay, in a given time, under varying pressures, through a capillary tube. Thus, the line of demarcation between a viscous suspension and a plastic substance is that the pressure-flow curve passes through the origin in the case of the former, but intersects the axis at a definite pressure value for the latter. The slope of the linear pressure-flow curve is a function of the mobility and the intercept of the yield value. Up to the present time definite plasticity values have not yet been obtained owing to difficulties with the capillary tubes (Hall [15]), but the method of attack is most promising. De Waele,[16] on the other hand, thinks that the relation which applies is: $P/Q = K$, for liquids, and $P/Q^n = K$, for plastic bodies, where $P =$ applied pressure, $Q =$ volume discharged and $n < 1 =$ degree of plasticity. Hence a plastic substance will become a viscous suspension when $n = o$, even though it may retain its solid appearance.

In the case of the clays in the plastic state we are dealing with water contents of from 15-50 per cent, according to the fineness and characteristics of the clay matter. Hence the amount of water required to convert dry clay into plastic material is a useful index of its possible plasticity. The working qualities of clay in this state are affected also by the presence of electrolytes but owing to the great internal friction to a much less extent than in the case of suspensions. Still the addition of OH ions reduces the plasticity to a marked extent. According to Rohland increase in acidity should tend to improve plasticity but this is not the case. Hence the effect of the OH ions is a far more potent one, which might be expected in view of the acidity of the clays. The effect of various electrolytes upon the same clay is very contradictory. Thus NaCl was found to decrease the drying shrinkage of a clay while Na_2SO_4 increased it. It is evident that the conditions are too complex to permit of generalizations.

The previous history of a clay, the weathering or soaking it has undergone or the mechanical treatment it has received are of profound influence upon its working qualities, in fact to such an extent that a thoroughly aged clay may bear no resemblance to its original condition. However, there are clays which are improved but little by storage. The effect of aging has variously been ascribed to the growth of bacteria, algæ and the increase in acidity. But it is more probable that it is a general breakdown of the coarser clay aggregates into finer ones and the more uniform distribution of the water content.

The volume of dry clay plus a given volume of water generally yield a volume of plastic substance larger by from 1 to 5 per cent[17] than the sum of the volumes of clay and water, due to mechanically enclosed air and perhaps to a slight swelling of the clay substance proper.

Immediately upon the molding of plastic clay evaporation of water begins which is accompanied by a shrinkage in volume. The shrinkage is exactly equal to the volume of water lost, down to the point at which further contraction ceases. The proportion of the total water thus evaporated is called the shrinkage water. The most plastic clays also show the greatest contraction. According to the nature of the clay the contraction may be from 10 to 35 per cent, in terms of the original, wet volume. The water remaining in the clay after it has reached its maximum shrinkage minus a small amount which cannot be expelled below 100°, is called pore water. The last portion of residual water which requires higher temperatures for its expulsion is the hygroscopic moisture.

The rate of evaporation of water from the clay is governed by the capillary flow from the interior to the surface. If in the case of highly colloidal clays the water is evaporated more rapidly from the surface than it can be supplied from the inner portion, stresses are produced which may result in visible or invisible cracking. The permeability of clay to water may be said to vary as K/r^3, where $K =$ constant and $r =$ mean radius of particles.

Properties of the dried clay. Completely dried clay will absorb moisture very eagerly from the atmosphere according to the humidity which prevails. The amount of water thus taken up may be said to be proportional to the amount of colloidal material present, *i.e.*, it varies inversely with the mean diameter of the particles. The quantity of water thus taken up may be as high as 6 per cent.

The dried clay which is more or less porous, according to the nature and quantity of bonding (colloidal) material, possesses a varying degree of mechanical strength and it reaches maximum strength when heated above 100°. This point is of considerable importance for many industrial purposes. Values of the strength of different dried clays are compiled in the following table:

Clay	Tensile strength lbs. per sq. inch	Transverse modulus of rupture lbs. per sq. inch	Compression lbs. per sq. inch
English ball clay	210	558	1148
English china clay	41	98	228
American ball clay	125	380	633
Fla. Kaolin	104	239	539
N. C. Kaolin	69	166	349
Georgia Kaolin	147	325	455
Penna. fire clay	155	508	631
Ohio shale	136	311	636

We have thus available clays of various mechanical strengths which we can adapt to our needs. In the drying of clays any defects due to faulty molding or to lamination planes become accentuated and may give rise to real or incipient fractures which may culminate in serious defects during the firing process.

It is interesting to note that excessively plastic and sticky clays when heated from 200-400° before being worked lose much of their plasticity and high shrinkage. Difficultly drying materials may thus be treated and rendered useful. This process also is quite promising for the treatment of clays which are to be cast since it renders them more suitable for this purpose. In the pre-drying treatment it is evident that the colloidal material is "set" reversibly.

Effect of heat upon clay. When clay is heated, beginning with 100°, the first effect noted is a distinct temperature lag and loss in weight up to about 225° due to the expulsion of the hygroscopic water. The weight then remains constant up to about 500° when another drop in weight is observed which, with a rising furnace temperature, occurs until 650° is reached. This loss in weight corresponds to the expulsion of the chemically combined water, a point which also marks the destruction of the original clay molecule. A number of interesting properties are associated with the dehydrated state which is practically irreversible —except under higher steam pressure. At this point the clay undergoes a distinct increase in volume which may be as much as 4.6 per cent of the initial volume. The loss in weight for pure clay substance is practically 13.9 per cent. Dehydrated clay is chemically very active since it combines very readily with alkalies and the hydroxides of the alkaline earths. The compounds formed with $Ca(OH)_2$ have the properties of hydraulic cement. The material is also much more soluble in acids than the raw clay. A further peculiarity of dehydrated clay is its catalytic activity both in inorganic and organic reactions. Thus, it promotes, in the presence of steam, the oxidation of SO_2 to SO_3. The material likewise is a powerful absorbent of steam and vapors and has decided desiccating properties. All of these characteristics are lost when the clay is heated to higher temperatures. The de-

hydrated clay has the highest porosity the material can attain. The dehydration is an endothermic process absorbing, according to Navias,[18] 240 calories per gram under the assumption that the steam is condensed to the liquid at room temperature. As to the reaction taking place Mellor and Holdcroft [19] maintain that it results in the formation of free alumina, silica and steam. On the other hand, Knote suggests [20] the reaction:

$$2Al_2O_32SiO_22H_2O \rightarrow Al_2O_3SiO_2 + Al_2O_33SiO_2 + 4H_2O.$$

W. and D. Asch consider that no dissociation of the silica-alumina complex occurs.

Coincident with and following the dehydration stage there takes place the oxidation of the various forms of carbon present as well as of the ferrous oxide and sulfides. This stage must be carried to completion so that no unoxidized materials are left behind. Failure to perform this operation results in serious faults having to do with the later evolution of gases which often cannot escape readily and hence cause the mass to bloat. Oxidation must hence take place while the porosity is great enough to provide means of ready escape of the gaseous oxidation products.

With the rise of the kiln temperature the clay begins to contract and to decrease in porosity, due in part to colloidal changes and partly to the incipient fusion or sintering of the most easily fusible constituents. At about 950° an exo-thermal reaction takes place which Mellor and Holdcroft ascribe to the condensation or polymerization of free alumina, Knote as well as Ashley to the formation of $Al_2O_3SiO_2$ and W. and D Asch to the polymerization of the clay anhydride formed at 500-600°.

With the further increase in temperature, shrinkage in volume and reduction in porosity may proceed slowly or rapidly according to the composition of the clay. If the material is essentially pure clay substance the curve correlating the contraction with temperature will show a slight slope. Given a constant rate of heating the closing up of the pores will proceed at the rate $\frac{dp}{dt}$ quite proportional to the kind and the amount of fluxes. In the case of the impure clays the curve will drop rapidly and approach the condition of minimum or zero porosity at a low temperature. We say then that the clay is vitrified. What happens is that partial fusion or sintering takes place with the formation of a glassy or slaglike phase the amount of which increases rapidly in the case of the impure and slowly in the case of the pure clays. It is evident also that the composition of this phase must change with increasing temperature, becoming more and more siliceous and aluminous. The contraction is caused by the effect of surface tension upon the

exterior surface of the clay mass to which the gradually softening material yields.

A curve showing the relation between the volume or the shrinkage of the clay, and temperature expresses the firing behavior of the substance in a very satisfactory way and graphs of this kind are used extensively. We must keep in mind also that the effect of the rate of heating is exceedingly important. The faster the heat is applied the higher must be the vitrification temperature, and vice versa. To understand this relation thoroughly is one of the requisites of correct firing.

The heat consumption involved in the complete firing of the purer types of clay, according to Navias,[21] at a finishing temperature of 1225° amounts to 600 calories per gram of dry material, or 0.5 calories per gram per degree.

It is evident that the viscosity or rigidity of the clay must decrease with increasing heat effect until the state of fusion is reached. Overfired clay hence tends to become distorted before this condition is reached and shows the characteristic surface tension effect evidenced by the rounding of the corners and edges.

Whether or not amorphous sillimanite is produced at a temperature as low as 950° it is a fact that the clay substance decomposes first into a non-crystalline or crysto-crystalline, and at higher temperatures into well developed, needle like crystals, which have been found to be mullite, $3Al_2O_3 2SiO_2$, by Bowen and Greig.[22] Assuming that mullite is formed the reaction would be: $3(Al_2O_3 2SiO_2) \rightarrow 3Al_2O_3 2SiO_2 + 4SiO_2$. The silica thus split off becomes part of the glass phase. In highly aluminous materials it is also possible that this reaction may proceed still further resulting in increasing amounts of corundum plus glass.

The development of the crystalline phase is of considerable importance with reference to the mechanical properties of the clay. In the case of coarse crystallization, according to Mellor, the mechanical strength may suffer or it might again be improved by recrystallization. The formation of minute crystals seems to be conducive to high mechanical strength. But it must be realized that with increased formation of mullite the proportion of the glass phase keeps pace so that the volume of more fusible material in which the crystalline matter, may be said to float, may have a tendency to flow under comparatively low pressures. The gradual lowering of the resistance to compression observed when clays are heated to higher temperatures shows the constant decrease in viscosity of the system. The lowered resistance to deformation at temperatures at or above 1350° under pressures from 25-40 pounds per square inch has been used in the testing of refractory clays.

Purdy has found that common with nearly all of the silicates the density of gradually vitrifying clays is lowered so that the drop in specific gravity in a way is a measure of the amount of heat work done.[23] At higher temperatures this increase in volume is counteracted

by any crystallization which may take place, depending upon the extent.

The most common accessory constituent of clay, quartz, exercises a profound influence upon all of the properties. It decreases the drying as well as the firing shrinkage, causes the clay to be more rigid under compression at furnace temperatures and makes it peculiarly subject to stresses in heating and particularly, in cooling owing to the inversion of alpha to beta quartz at 575° and also the inversion of alpha to beta cristobalite at about 230°. The volume changes [24] involved are readily computed from the specific gravities of the different forms which are: alpha quartz, 2.65; beta quartz, 2.633; alpha cristobalite, 2.333; beta cristobalite, 2.21. The cristobalite inversion is particularly important in the case of glazed products, since the transformation occurs at a point when the glaze is rigid and cannot yield to the change in volume as it would at higher temperatures.

In exceeding the proper maturing firing temperatures of clays we find that they "over fire," in that they evolve gases which originate from the decomposition of sulfates, the change of ferric to ferrous oxide or magnetite and the setting free of dissolved gases. Hence the exterior volume increases and the structure becomes vesicular. As a rule the usefulness of a clay is destroyed when this stage is developed. It is necessary hence that the heat reactions be arrested before this point is reached.

The final fusion of the clay is of no commercial interest although the softening point must be known in the case of refractory clays. It must be clearly understood that there is no such thing as a definite melting point of clays but that owing to the high molecular friction there is only a gradual softening, dependent both upon temperature and time. In the case of the purest clays the softening point may be as high as 1750°. Impure materials may fuse at temperatures as low as 1100°. In the absence of basic fluxes an increase in alumina invariably raises the softening temperature, forming mullite which melts at 1810°, splitting off corundum. There is a eutectic between cristobalite and mullite which melts at 1545°.

Properties of clay in the fired state. It can readily be seen that the physical properties of clays in the fired state must vary enormously both according to their physical and chemical characteristics and the treatment they have received. It is impossible, therefore, to give any average values, especially since results are lacking for many clays and a number of physical properties.

The compressive strength at atmospheric temperature may vary from 2000 to 40,000 pounds per square inch; the modulus of rupture from 500 to 6000; the modulus of elasticity from 500,000 to 7,000,000; the tensile strength from 500 to 5000 pounds per square inch. No claim is made that these limits are exact.

Similar variations occur for other physical properties, such as re-

sistance to impact, abrasion, torsion and penetration in reference to which there is a scarcity of determinations. The same thing is true also for the thermal properties. The coefficient of thermal expansion may vary between wide limits according to the degree of firing which the material has undergone. Thus, the mean linear coefficient for a kaolin [25] may vary from 419×10^{-8} at 1150°, to 520×10^{-8} at 1250°; for a dense ball clay from 729×10^{-8} at 1090° to 1079×10^{-8} at 1250°. Attention might be called to the far reaching effect of quartz in this respect with a linear coefficient of 1660×10^{-8}. It must be realized that even for the same material fired at a given temperature the coefficient of thermal-expansion varies at different temperatures and is not necessarily a linear function but is very apt to show maxima and minima. For most purposes we are chiefly interested in the thermal expansion between 20-600°. In making such measurements care must be taken to differentiate the expansion caused by the transformation of alpha to beta quartz from the pure thermal expansion. A great deal remains to be done in this field and a number of contradictory points must be cleared up. The resistance of clays to sudden temperature changes is connected with the heat expansion and the diffusivity of these materials.

The specific heat of burnt clay increases with temperature. For clay fire brick the following relation has been found by Bradshaw and Emery:[26] $S = 0.193 + 0.000075\ t$, where t = temperature, degrees C.; S = specific heat. Usually these values vary from 0.200 to 0.235 for temperatures up to 1000°, but specific heats as high as 0.263 have been found at the latter temperature.

The thermal conductivity of clay likewise increases with increase in temperature. Thus, the thermal conductivity of fire clay bricks was found by Wologdine [27] to vary from 0.0035 gram calories per cm.^{3} per second, at 1050° to 0.0042 at 1300°. The thermal diffusivity of a burnt fire clay body was found by Green to vary from 0.0017 at 500° to 0.0026 at 1100°.

Dougill, Hodsman and Cobb [28] suggest the following relation between temperature and thermal conductivity:

$$Kt = 0.00155 + 0.25 \times 10^{-5}t,$$

where Kt = thermal conductivity of clay fire brick, in gram cals, per sec. per cm^3., at $t°$.

The electrical resistivity of clays, while high at low temperatures decreases rapidly at higher heats, according to the amount of fluxing material present. Thus Henry [29] found the resistivity of North Carolina kaolin to be 133×10^5 ohms per cm^3. at 300° and 106×10 at 1300°.

The Testing of Clays. Although many tests of clays are constantly being reported a great deal of such work is incomplete. The

minimum requirements of a clay test should include the following determinations as far as they relate to the general properties. For specific purposes additional information is necessary.

1. *Structure of clay*

Determination of the general structure, whether hard or soft, coarse or fine grained. The rate of slaking of the clay in water if it is not of such a nature that it must be ground, should be found.

2. *Water content of the clay in the plastic, most workable state.*

This is determined by weighing the plastic clay, drying it in the air and finally at 110° and weighing it. Difference in weight is equal to water content and is to be expressed in per cent of the wet weight.

3. *Drying shrinkage*

The volume of the plastic clay specimen is measured by means of a suitable voluminometer, using petroleum as the displacing liquid. Similarly, the volume of the dried specimen, saturated with petroleum, is determined. The difference in volume is the drying shrinkage and is expressed in per cent of the wet volume. The volume shrinkage must always be accompanied by the water content of the clay in the plastic state to make the value at all useful.

4. *Transverse strength in the dry state*

Bars, usually 6′ x 1′ x 1″, made from the clay, then dried in air and finally at 110°, the cooling being done in desiccators over sulfuric acid, are tested for transverse strength and the modulus of rupture determined in the usual manner.

5. *Porosity and firing shrinkage*

Suitable test specimens, after drying, are fired to a series of temperatures, usually not less than six, and are withdrawn from the furnace at definite points from pyrometer readings or as indicated by pyrometric cones. These are measured for porosity and volume shrinkage. The porosities may be computed from Purdy's formula:

$$P = 100\,\frac{w-d}{w-s},$$

where P = porosity, in per cent.
w = weight of specimen, saturated with water.
d = dry weight.
s = weight of saturated specimen, suspended in water.

The work may be simplified by employing either the porosity or the volume shrinkage. The results are plotted to show the graphical relation between the temperature, porosity or volume shrinkage.

6. *Strength in the fired state*

Bars, made from the clay, fired to one or more temperatures are tested for transverse strength. The selection of the proper temperatures depends upon the kind of clay and the purpose for which it may be used, according to the previous tests. This is a matter to be decided by the judgment of the operator. In connection with the results of this test the porosity of the specimens should be given.

7. *Color*

The color of the clay, fired to different temperatures must be described.

8. *Softening point*

In the case of all clays intended for refractories as well as for most other materials it is desirable to determine the softening temperature. This is the temperature at which a small tetrahedron of the material definitely deforms.

According to the purpose for which the clay appears to be suitable it may be necessary to determine its fineness by means of the mechanical analysis, supplemented by the determination of the amount of dye adsorbed from a 0.3 per cent malachite green solution, which affords an index of the fineness. It may be desirable also to determine the true porosity of the fired material from the apparent density and that of the powdered substance, or the resistance to impact, abrasion, compression at atmospheric or furnace temperatures, or tension. It may likewise be advisable to determine the thermal expansion of the clay, its resistance to sudden temperature changes, its thermal conductivity or diffusivity. Finally, in special cases, it will be necessary to determine the electrical resistivity. These supplementary tests are often of great importance in fixing the best use to which a clay can be put.

For the many details and the special information necessary for a proper understanding of the properties of clays the reader is referred to: The Chemistry and Physics of Clays, by A. B. Searle.

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BENTONITE

By Jerome Alexander

Definition. The most satisfactory definition of bentonite thus far proposed is that by R. B. Ladoo (Publication No. 2289, Bureau of Mines, 1921): "A group or series of clay-like materials characterized by an alkaline oxide and alkaline earth content of 5 to 10 per cent, fine grain size, high adsorptive powers, and usually very strong colloidal properties." The name was assigned to this material from the locality where it was first discovered, Fort Benton, Wyoming, but many occurrences have since been recognized in various parts of the United States and Canada.

Properties. The color of bentonite varies from white to cream, gray, pink, brown, or even black, depending on the nature of impurities which chance to be present. Freshly cut surfaces of the clay, as taken out of the ground, usually show a waxy luster, but as moisture is lost on exposure to air it becomes dull gray. Its color when fired is white, buff or brown (Schurecht and Donda, *J. Am. Ceramic Soc.* 6, 940, 1923).

Bentonites vary still more strikingly in colloidal properties, samples from Tennessee which I have examined being practically non-colloidal, but others from Wyoming, California, etc., exhibiting under the ultramicroscope a large percentage of particles of colloidal dimensions, showing active Brownian movement. The more colloidal bentonites exhibit astonishing powers of absorbing water, and swelling to a jelly-like mass, some varieties then occupying six to eight times their original volume. On exposure to the air they soon shrink to a powder again, and the surfaces of the deposits show a curious aspect as a result of this, being during dry weather dry and fluffy, but after a rain becoming covered with a thick mass of very slippery jelly.

The following microscopic observations on aqueous suspensions are reported by Dr. L. C. Hazen (Private communication):

Wyoming sample.—Particles range from ultramicroscopic to 4-5 microns, the majority being from 0.1 to 0.5 microns.

California sample.—Limits of size narrower than the preceding, the general run being about 0.1-0.2 microns, with none over 1 micron, and fully half ultramicroscopic, or just within the range of vision of an oil immersion objective.

Dr. Hazen found that 0.85 per cent sodium chloride solution causes

rapid flocculation and sedimentation, and remarks that this is an interesting case of the effect of electrolytes on colloidal suspensions.

Schurecht and Douda (*loc. cit.*) report the following points of interest in the ceramic field: softening point, cone 1 to 30; per cent water of plasticity, 22.07-114.61; per cent volume shrinkage (in terms of dry volume), 24.21-195.81. They found that the addition of 32 parts of bentonite to 50 of flint and 50 of kaolin ran the modulus of rupture from 43.87 to 319.37 lbs. per sq. in.

Occurrence and origin.—The principal commercial deposits of bentonite are found in South Dakota, Wyoming, and California, but there are deposits of varying size and quality in Tennessee, Texas, Arizona, some of the western Canadian provinces, and elsewhere. Its mode of occurrence and certain of its features clearly indicate that it represents a decomposition product of volcanic ash. This seems to have been first pointed out by Hewett (*J. Wash. Acad. Sci.* 7, 196, 1917) but was discussed in greater detail by Wherry (*ib.*, 576-583). The evidence comprises such things as the essential absence of quartz (which is present in all ordinary clay sediments) the presence of feldspar, and the extension of individual beds, often very thin ones, over vast distances without important variation in thickness. The clayey matter in normal sediments has been so acted on by electrolytes and other active substances that it has lost its original porosity and tendency to take up water, accompanied by swelling, which bentonites exhibit to such a marked degree. The chemical composition of the bentonite is, moreover, exactly what would be expected from the partial alteration of an intermediate igneous rock, accompanied by the removal of alkalies and addition of water. While the beds of bentonite are often of enormous extent, the Tennessee deposits, for instance, being estimated by W. A. Nelson (*Bull. Geol. Soc. Am.* 33, 605-616, 1922) to extend over an elliptical area about 800 miles long by 450 miles wide, recent volcanic eruptions such as those of Tomboro, Krakatoa and Katmai are known to have ejected many cubic miles of ash material.

The following summary of the formation and nature of bentonite was prepared at my request by Dr. Edgar T. Wherry. It contains certain extensions of his views reported in the paper above cited, especially in the recognition of a definite mineral as present in large amount in the bentonite rock (a rock being a mixture of minerals) and in the pointing out that many of the particles of this substance appear to be of visible dimensions in two space-directions and of colloidal dimensions in the third direction in space.

"A volcano explodes and sends up into the air a vast body of pulverized glassy lava, which is carried off by the winds and deposited in strata, mostly thickly near, but thinly even hundreds of miles from the source. In the case of the South Dakota and Wyoming deposits, the sources were probably in the middle Rocky Mountains; in that

of the Tennessee deposits, the volcano seems to have been under the present Cumberland plateau. With this lava dust are carried more or less hydrochloric acid, hydrofluoric acid, sulfur dioxide, and perhaps other corrosive gases. These attack the surfaces of the particles of lava, rendering them open to relatively rapid disintegration by such weathering agencies as may effect them. When the lava-dust falls on dry land, and the climate is dry, as in Nebraska, the particles may remain fresh and unweathered for whole geological periods, as in the case of the "volcanic ash" deposits worked commercially for Old Dutch Cleanser, etc. But when the lava dust falls into regions of moist climate, or into bodies of water, it may undergo rapid weathering and disintegration.

"The original lava is glassy and amorphous, and the first product of weathering or disintegration is likewise amorphous, but consisting largely of alumina and silica in partially hydrated form, it also possesses many of the properties of colloids. Some of the bases in the original lava are dissolved in the rain or other water, and carried away, but a part of them is adsorbed by this colloidal matter.

"In time the colloidal gel starts to become crystalline, a large number of nuclei developing in it, and crystal material building around these nuclei until the whole mass is occupied by minute crystalline grains in contact with one another; it is then of the character to which I have assigned the name *'metacolloid,' i.e.*, a colloid which has become crystalline in place, without intervening solution and recrystallization. Such colloids turned into metacolloids are common among minerals, as for instance chalcedony, limonite, psilomelane, and many clays. The nature of the crystalline substance depends on the original composition of the colloidal gel. In the case of the better known deposits of bentonite, the lava seems to have been originally of intermediate composition, that is, containing 60-65% SiO_2 and 20-25% $Al_2O_3 + Fe_2O_3$. In the resulting colloid, the ratio of Al_2O_3 to SiO_2 is near 1:2.

The mineral tending to form is a member of the mica group, and possesses the eminent basal cleavage of the micas, a cleavage which has been shown to extend down to single molecules (by tearing off thin flakes with a bit of hot selenium, and determining their thickness by interference phenomena). When a crystallized mineral of such structure forms as a metacolloid, the individual crystalline grains are likely to be extremely thin; and it seems to me that the properties of bentonite indicate that in it the thickness of the crystals is of colloidal dimensions, although in length and breadth they are of microscopically visible size.

"Under the microscope, between crossed nicols, the grains show distinct double refraction, which I formerly thought a strain phenomenon in a colloid, but now agree with Dr. Larsen in recognizing as evidence of crystallinity. When enough of these grains or flakes overlap, interference figures can even be seen. The material swells up in water to

a surprising degree, becoming highly plastic; the change in appearance between crossed nicols suggests that water is taken up by capillary attraction, spreading the grains apart; while their excessive thinness (colloidal dimensions!) renders them capable of plastic flow. It seems to me that the industrially valuable properties of bentonite, such as water-softening, are connected with this peculiar relation of dimensions,—the grains being of visible dimensions in two directions, and thus being capable of attaining a high degree of solidity, yet of colloidal dimensions in the third, and thereby exposing the usual enormous surface per unit weight characteristic of colloids, and so exhibiting marked adsorption properties."

While Dr. Wherry's view that bentonites contain particles of what may be termed "one-dimensional colloids" may well be correct, the bentonite seems not to have passed entirely into this form, for my ultramicroscopic study shows the existence of many particles of colloidal size. No doubt different samples show variation in the relative proportions of the wholly colloidal, and one-dimensional colloid particles. At any rate some such explanation is necessary to account for the enormous powers of water absorption and swelling possessed by the material, it far exceeding any ordinary colloidal substances in this respect.

Composition.—Bentonites vary widely in chemical composition, this feature being less significant than their physical character. To give some idea of their general character, however, the analysis of one from Belle Fourche, S. Dak., is given here, together with maximum or minimum values of some of the constituents shown by samples from other deposits:

	Belle Fourche	Other Deposits
SiO_2	60.64	54.00
Al_2O_3	23.26	11.84
Fe_2O_3	3.92	3.97
CaO	0.59	4.94
MgO	2.19	3.24
Na_2O	4.33	0.66

(The balance is water and small amounts of other constituents.)

Uses.—Notwithstanding the valuable properties of the more colloidal and highly adsorptive varieties of bentonite, as well as its cheapness, marked efforts have only recently been made to find places and ways in which it can be used. As is usual with an unfamiliar material, some find out how to use it where others fail, the result being that many claims are made as to what it will do, but some of them cannot be verified by other experimenters.

One of the first uses of bentonite was for stuffing horses' hoofs, and it was sold under the name of "White Rock" and the like. Made up

into a jelly with water, it forms a cataplasm or poultice that has great power in reducing swellings and inflammation, and draws the soreness from the horse's foot. It has been used to advantage in cases of rheumatism, and seems to act similarly to the so-called *fango baths* or mud baths, for which many people go to Europe. It has some value as a "beauty clay," even if it will not convert a home favorite into a movie queen. It is said to be the basis of the proprietary poultice material called "Antiphlogistine."

In the ceramic field it has large possibilities. M. E. Manson (*J. Am. Ceramic Soc.*, July, 1923) indicates that it may be used to suspend enamels. Using 1% bentonite instead of 2½% of clay in grinding a frit for making a ground coat, he found that the mixture stayed in suspension much better than it had ever done before. Paul E. Cox (*J. Am. Ceramic Soc.*, *7*, 151 (1924)) studies plasticity by practical-potter's methods, and states: "Bentonite added to some mineral mixtures will produce plasticity. This plasticity is the exact plasticity found in clays liked by the practical potter. North Carolina kaolin mixed with bentonite can be made to have all the plasticity of a high grade ball clay." Since a small percentage of colloidal clay will markedly affect the properties of a soil, it seems that only a little bentonite is needed in most pottery mixtures; so that the use of larger amounts than are demanded will cause trouble. It should also aid soils that are deficient in colloidal matter.

Bentonite can also be used as a filler in soap. I have found that it gives toilet soap a particularly velvety feel, and judging from the fact that clay soaps were widely used in Germany during the war, it must have some detergent value. Indeed, locally it has been used as a soap, and extravagant claims were made for it. Its value must be worked out in each case, but from the fact that it has great adsorptive powers, and that its particles have active Brownian motion, there is some basis for the claim that it aids in laundry soap.

According to the Bureau of Mines, experiments at the Forest Products Laboratory seem to show that the addition of small amounts of bentonite to the clay used in filling paper greatly increases the percentage of clay held by the finished paper. The following are some of the results:

Clay %	Bentonite %	Retention %
90	10	54
80	20	64
0	100	84

It is said that the bentonite gives a superior feel to the paper, and because of its smoothness, bentonite should be valuable in paper coating mixtures.

The Forest Products Laboratory has also found bentonite valuable in de-inking news paper, for it absorbs the ink without affecting the pulp. It is suggested for use as a filler in phonograph records, cordage, textiles, molded compositions, and rubber, where its enormous free surface should make it valuable. Since it readily adsorbs many aniline colors, it can be used as a color base, or as an addition to other bases, and can be used in paints, and calsomines.

It is said to be an excellent retarder in plaster of Paris, but on trial, I found that it *increased* the speed of set of a commercial plaster of Paris, possibly because it adsorbed the organic retarder this contained. It is used as a good bonding material for electrical and chemical porcelain graphite crucibles, abrasive wheels, etc. Some varieties, when burned and granulated, are used as water softeners, acting as zeolites.

In U. S. Patent 1,286,043, Leon McCullogh of the Westinghouse Electric and Manufacturing Company covered the use of a bentonite paste as a temporary binder for mica flakes, punching being made before complete drying. U. S. Patents 1,365,331 and 1,386,008 record further improvements in the manufacture of electrical insulation involving the use of bentonite as a binder or assistant binder.

W. A. Nelson (*loc. cit.*) states that boiling bentonite with $5NH_2SO_4$ destroys its colloidal properties, about 85% of its Al_2O_3 going into solution as aluminium sulfate. "This fact offers a possible solution to the origin of bauxite, as laboratory experiments show that such a solution of aluminium sulfate is precipitated by a solution of tannic acid after standing for a few days. Deposits of bentonite occurring in contact with pyritiferous rocks would readily have part of their aluminium contents dissolved out by the sulphuric acid in the ground water, and such a solution of aluminium sulfate, it seems, would be precipitated by natural reducing agents, such as tannic acid or acid peat-forming bacteria, and thus form certain of our present bauxite deposits." In another paper (*Bull. Geological Soc. America, 34,* 525-540 (1922)), Nelson gives further data supporting this view.

U. S. Patents 1,408,655-6 granted to C. W. Stratford, describe the preparation and use of bentonite clays and the like in the refining of petroleum. Certain varieties have shown 16 times the efficiency of Fuller's earth for this purpose. The patent covers only the continuous process of preparing the clay by washing, treating with sulphuric acid, and drying; this removes the alkalis, alkaline earths, and some of the alumina, leaving a residue of very fine grains of aluminium silicate.

Bentonite is a valuable emulsifier, and in effect acts as a protector. The pioneer work of S. U. Pickering (*J. Chem. Soc. 91,* 2001, (1907)) found that many insoluble substances would emulsify oils in water, and bentonite is one of this class. It should serve for emulsifying cutting oils, stuffing oils for leather, and many similar purposes. An im-

portant application of bentonite as an emulsifying agent has been made by L. Kirschbraun, who emulsifies with it asphalts, pitches, etc. These emulsions may be used as paints, for roofing, for the protection of structural steel, etc., and also added to paper pulp in the beater so as to produce a wide variety of waterproof papers.

Because of its bodying properties, bentonite of the proper kind should be of use in thickening pastes, sizings, printing gums, shoe polishes, and many other things. Since all mixtures of this character vary greatly in ingredients and in their conditions of use, common sense, good judgment, and some experience are necessary if the best results are to be expected.

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PLASTICITY IN COLLOID CONTROL

By Eugene C. Bingham

In crystalline materials, properties such as the melting-point, boiling-point, fluidity, and solubility have proved to be invaluable, but in non-crystalline materials, many of which are soft solids and colloidal in nature, these properties are either lacking altogether or are ill-defined. A single example will suffice to illustrate this statement. Dental impression waxes are made up of varnish gums, linseed oil, stearic acid, talc, coloring matter *et cetera.* In spite of the use of non-uniform materials, it is of the utmost importance to secure uniform properties of a certain sort which are capable of description and presumably of measurement. The material must take a very exact impression of the patient's mouth at temperatures which can be tolerated by the body, and yet at the temperature of the body the material must be rigid enough for removal without distortion. Chemical composition in this case means nothing, and the melting-point, boiling-point and solubility cannot be determined to advantage. It is quite obvious that since the properties of flow are involved, they should be the ones used for control. But even here a caution must be added that one must not expect useful information from determinations of the "apparent viscosity" [1] if the material dealt with is really plastic in character. There is therefore a need for new control properties particularly for that class of substances which we commonly think of as colloidal.

There are many cases like the above in which the properties of flow are important. The following list is not exhaustive but it may be suggestive.

1. Paint
2. Lime and plaster
3. Fondant
4. Clay
5. Enamels
6. Dyes
7. Carbon black
8. Asphalt and bituminous materials
9. Surface films
10. Metals and alloys
11. Slags
12. Cements
13. Rocks in the earth's crust
14. Starch
15. Cellulose
16. Cellulose nitrate
17. Cellulose acetate
18. Viscose
19. Condensation products
20. Soaps
21. Fats and greases
22. Chocolate, and condensed milk
23. Rubber
24. Gums and varnish
25. Gelatine and glue
26. Leather
27. Gluten and flour
28. Blood and humors
29. Glass
30. Lubricating oils

[1] *Fluidity and Plasticity*, McGraw-Hill Book Co., pp. 52 *et seq.*

Too much has there been a tendency to measure not flow itself but something else supposed to be related to the flow. Thus the absorption by clay of malachite green has been used as a measure of plasticity. The shrinkage of clay on drying or on burning have been supposed to also measure plasticity but none of these has given an absolute measure of plasticity or can the various tests be properly correlated.

Much attention has been given to the so-called hardness test, particularly as applied to metals. In this test plastic flow plays a most important rôle, yet scarcely any effort has been made to express the plasticity of metals in absolute units and thus sharply define the properties of hardness, malleability, ductility, elastic after-effect, ultimate strength and the like.

A great amount of time has been spent, one might almost say misspent, in the effort to determine the "melting-points" of materials like paraffin and butter, which have no true melting point such as we know in the case of crystalline pure substances. In most cases the method depends upon obtaining a temperature at which a certain amount of flow has taken place under carefully specified conditions. But there are many methods and the specified conditions are different in each one, so the "melting-points" are different and if we can accept the authority of Lewkowitsch, they are all nearly worthless.

Again it can be shown that the solubility of colloids depends not upon the conception with which we are familiar in crystalloids, but upon the plasticity of the dispersion. Acetone is a good "solvent" for nitrocellulose because it forms a readily mobile dispersion. Other examples might be cited to show that the importance of the flow of colloids has not been appreciated. Such efforts as have been made along this line have led into a morass of complexity instead of out into the clear light. Thus Glaser clearly demonstrated that which was contrary to all experience with true fluids, viz. that the viscosity of a colloid may vary widely with the shearing stress. Others, perhaps very many others, made this same discovery, but no one realized its possible importance.

Whatever may be the difficulties connected with the measurement and the interpretation of the flow of colloids, it now appears certain that the effort should be made to express the plasticity of colloids in absolute units. Even were it found later that the problem is impossible of solution, it would be instructive to find out why this is so.

A variety of methods have already been tried out or suggested for the measurement of plasticity in absolute units. For rather mobile substances, the capillary tube, the rotating cylinder and the falling ball are available. For substances of higher consistency it may still be possible to extrude the material through small tubes after the method of Tresca or where this fails, the method of torsional rods or bending beams can be employed. There is therefore no *a priori* reason why the plasticity method should not be applied to all solids as well as semi-solids

and colloidal solutions. The unfortunate thing is that so little has been done to develop these methods and to correlate them. The author and his co-workers have devoted their attention almost exclusively to the development of three variations of the capillary method. It is greatly to be hoped that the number of methods available may be extended and the reliability of the methods improved. A method may be both slow and cumbersome and yet be of great value if by means of it we can obtain information which would otherwise be inaccessible. We may assume that quick relative methods will be developed later when we understand the fundamental theory of plastic flow. At present our knowledge of this theory is very defective.

It will be useful to review very briefly the present state of our

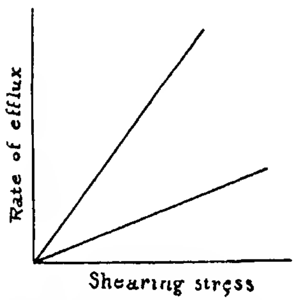

Fig. 1.—*Typical Fluidity Curves.* Two capillaries of different radius give different slope, but the curves are linear and they pass through the origin.

knowledge of viscous and plastic flow. We know that if a substance when subjected to shearing stress, were not to suffer permanent deformation Hooke's law[2] $s = F\ r$ would hold exactly. It does not hold exactly with any known material so there is some justification for the generalization of Heraclitus who claimed that "everything flows."

The fundamental relation of viscous flow[3] ($v = \varphi F r$) states that the deformation in each unit of time is directly proportional to the shearing stress (Fig. 1). It appears to be valid from the very smallest shearing stresses up to the point where the flow becomes turbulent provided that suitable corrections are applied for kinetic energy losses, end effects and so on. It is perhaps the uncertainty of these corrections that makes them unmanageable in turbulent flow.

In suspensions there is evidence (Fig. 2) that the relation[4] $v = \mu(F-f)r$ holds over a considerable range. Unfortunately the

[2] The shearing stress F dynes per square centimeter is assumed to deform the particles in a given plane a distance *s* in respect to another parallel plane at a distance *r*. The coefficient of elasticity is *ε*.

[3] The fluidity ϕ is the reciprocal of the viscosity η and v is the velocity, equal to s/t.

[4] The mobility, μ, the reciprocal of the consistency ξ. The yield value is *f*.

corrections for this type of flow are not yet thoroughly understood and with wide tubes and particularly at low shearing stresses the flow-stress curve is not linear. Since the difficulty can be obviated by the use of long capillary tubes, it seems reasonable to hope that with the

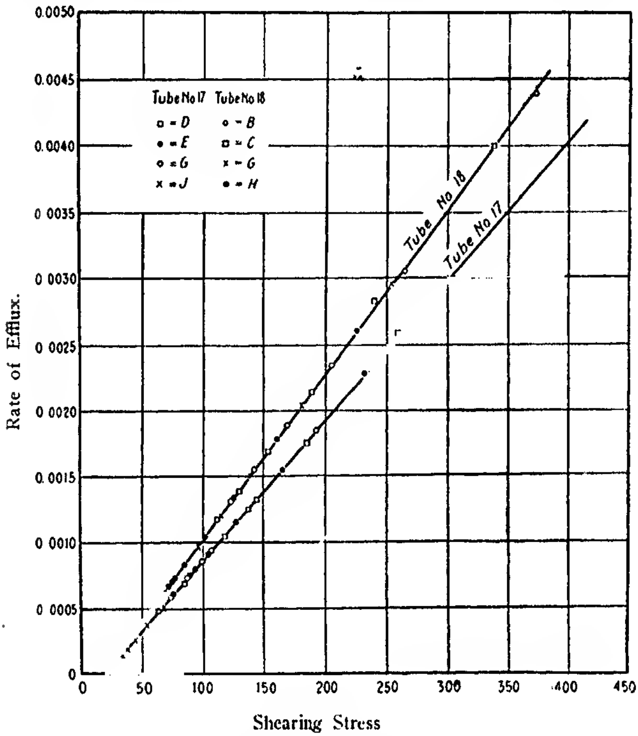

FIG. 2.—*The Plasticity Measurements on Lithopone Paint.* Two capillaries were used on the same paint and several series of measurements, indicated by the different letters, were made. In this type of material the curve is apparently linear and the yield value is independent of the shearing stress or the dimensions of the capillary.

evaluation of the *seepage* and *slippage* corrections the above formula will be found to be exact over a wide range of shearing stresses.

But notwithstanding that clear and indubitable evidence has now been found that in suspensions the flow is a linear function of the shearing stress and the yield value obtained is quite independent of the dimensions of the instrument, nevertheless in colloids of the emulsoid

type evidence is found for exactly the opposite conclusions. The flow of emulsoids through a long capillary is not a linear function of the shearing stress and the yield value cannot be obtained by simply extrapolating the flow-stress curve, Fig. 3, for with capillaries of different *radii* non-concordant values for the yield value would be obtained. This evidence has been obtained by Mr. Hood, Mr. Arnold and the author

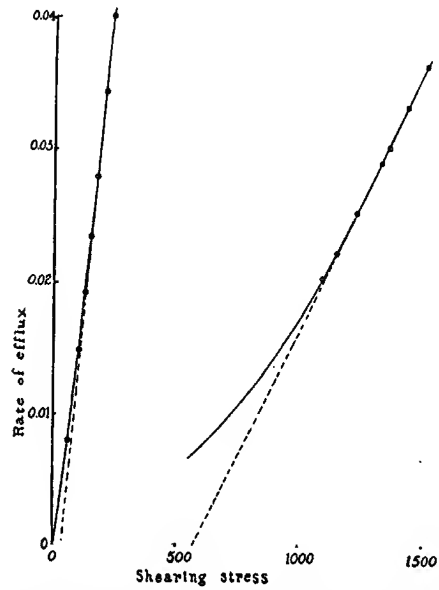

FIG. 3.—*The Plasticity Measurements on Nitrocellulose Solutions by Bingham and Hood.* The efflux-stress curves are not linear showing that the apparent yield value varies with the shearing stress. It also varies with the dimensions of the capillaries as can be seen by comparing the results from the two tubes.

and has not previously been presented. It will be presented in full elsewhere, although it would be out of place here.

To explain this sharp distinction between colloids of the two types it is necessary to examine into the causes of internal friction. In fluids it is recognized that internal friction arises primarily from two causes; the interdiffusion of molecules having different amounts of translational energy (diffusional viscosity) is very important in gases whereas in liquids the primary cause of internal friction is in the collisions between the molecules (collisional viscosity) caused by an actual spacial interference as the layers are sheared one over the other.

The viscosity of any fluid η is the sum of the diffusional viscosity η_d and the collisional viscosity η_c

$$\eta = \eta_d + \eta_c$$

and so far as is known this relation holds even through the critical state. In liquids far removed from the critical temperature the diffusional viscosity is of little importance and the viscosity is almost entirely collisional, following Batschinski's Law that the fluidity is directly proportional to the free volume. $\varphi = C(V - \Omega)$. The limiting volume Ω is the volume V which a mole of the liquid would occupy if the fluidity were reduced to zero. The free volume $V - \Omega$ is therefore the excess of the molar volume over the limiting volume. This gives one an exceptionally clear picture of the fluidity relations in liquids. From it one could predict the behavior of liquids under heat or pressure, for since heat generally causes liquids to expand considerably the fluidity would be expected to increase; and since pressure slowly decreases the volume a decrease in fluidity would be looked for. These expectations are realized.

But there is an important qualification which in no way invalidates the law of Batschinski. When there is a change in chemical combination brought about by heat or pressure or other cause, the molecular weight is changed and Batschinski's Law does not apply in its present form. This is most disconcerting in connection with associated liquids. If, however, the association is known the limiting volume may be calculated from the atomic constants.

In suspensions there appears a third cause for internal friction in that the microscopic particles in the shearing process are rotated. This disturbs the linear character of the flow and when two or more particles approach each other their rotation is stopped during the "collision" and a miniature "structure" is produced. Energy is continuously absorbed from the external shearing stress in breaking down these recurring structures. The fact that a certain shearing stress is required to overcome the yield values suggests that we are here dealing with ordinary static friction between the particles.

We might suspect that Batschinski's Law would apply to suspensions and this is found to be the case. The limiting volume becomes the concentration at which the mobility reaches its zero value. The medium filling the pore space in the closely packed material does not give the material any mobility but any excess over and above that needed to fill the pore space contributes to the mobility in direct proportion to its amount. Moreover the mobility is directly proportional to the fluidity of the medium. But anything that will cause the particles to adhere together as in flocculation will increase the yield value. On the other hand a mere change in the fluidity of the medium is without

effect on the yield value. The yield value does increase with the concentration of the disperse phase and in a linear manner at the higher concentrations.

Colloids of the emulsion type lower the mobility of the medium with extreme rapidity, one per cent of colloid lowering the mobility to a small fraction of its former value. This extraordinary phenomenon is generally accounted for by the disperse phase precipitating out in an insoluble network of some sort which acts like the reinforcing in concrete in preventing the shearing of one layer over another. If the interference were complete, there would be no flow, but quite contrary to what happens in the case of suspensions where the particles are not broken apart in the process of flow, the structure is continually breaking down in shearing the emulsoid. The tensile strength of the network will be more often exceeded with high pressures and wide capillaries because the shearing stress will then be greater. It appears therefore that in emulsion colloids it is necessary to introduce another property in addition to the yield value and mobility which is somewhat analogous to the association which we have in pure liquids and will take into account the resistance to breaking down in the structure of the liquid. Work on this subject is being continued.

In control work it seems not to be realized that fluidity is capable of very precise measurement. There is no difficulty in reaching a precision of one-tenth of a per cent particularly in relative measurements and fluidity in nature varies between the widest limits, viz. from zero to infinity. Errors of many per cent have been common in technical viscometry, but with the development of the theory of flow the highest precision will be advantageous in many places which need not be suggested here. The identification of substances by the plasticity method is particularly advantageous, because the yield value and mobility are independent variables so that an imitator would find it practically impossible to adjust both properties simultaneously.

The much discussed properties such as melting-point, solubility and hardness get a new meaning and an added importance through the plasticity method since they may now be defined and accurately measured as has not heretofore been the case. The temperature of zero yield value is the analogue of the melting point in colloid systems. As these so-called melting points are ordinarily determined the temperature is slowly rising or falling while the melting or freezing is going on. There is a very great hysteresis effect in very viscous materials which can be readily explained and it very greatly affects the determination, so that it is not uncommon for the freezing point and the melting point of colloids to be many degrees apart. By the use of the plasticity method this difficulty can be obviated because as many determinations as desired may be made at or near the transition temperature. Not only can the temperature of zero mobility be determined but the whole region of

softening can be explored. In studying mixtures made up of several components, several discontinuities in the plasticity curves may be expected.

The yield values for a given colloid dispersed in a variety of media at the same temperature, furnish a measure of the peptizing power of different media which is quite analogous to the solubility of a crystalloid in various solvents. The peptizability of colloids like solubility varies with the temperature.

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THEORY OF ADSORPTION AND SOIL GELS

By Neil E. Gordon

There are few fields in which more work has been done than that of soil chemistry, and adsorption is one of its principal phases. The importance of the soil being able to take up and release the soluble salts which are necessary for plants, can hardly be overestimated. Since the time of Van Bemmelens [1] much of the soil adsorption has been attributed to colloids, but the origin of these soil colloids and the rôle which each colloid plays in plant nutrition work, has been a subject of much speculation. The purpose of this paper is to give a picture of the colloid as it exists in the soil, and show the part each colloid plays in adsorbing, and releasing the soil salts.

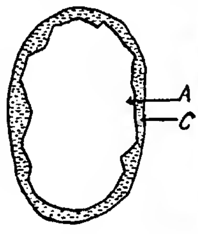
Origin of Soil Colloids

Some [2] believe that the soil colloids have resulted from the molecules of water bombarding the soil particles when the particles have reached the diameter of 0.0001 mm. This is one way of viewing their origin but our work at the University of Maryland gives us quite a different viewpoint.[3] All know that many soil particles are hydrated silicates which contain varying amounts of the alkali and alkali earth metals in addition to varying amounts of aluminum, iron and silicon. Soil chemists claim that these soil particles are surrounded with a water film which is held rather tenaciously. This would mean that the salts in the outer layer of the silicate particles are constantly subjected to hydrolysis. The sodium, potassium, calcium, magnesium, and other soluble salts would have a tendency to go into true solution while the hydrolytic products of the iron, silicon and aluminum salts, would result in an insoluble colloidal gel which would act as an incasing for the soil particles. Figure I might illustrate the appearance before this hydrolytic action has taken place, while Figure II would illustrate it after such hydrolytic action.

The soluble salts would partition themselves between the incasing gel and the surrounding water film. An equilibrium would be set up as in an ordinary adsorption experiment where the amount adsorbed

[1] *Van Bemmelens Landw. Vers Stat.*, 23, 265 (1879).
[2] Whitney, *Science*, 54, 656 (1921).
[3] Gordon, *Science*, 55, 676 (1922).

varies with the concentration of the solute. The respective adsorption power of these hydrolytic incasing products of silica, alumina, and iron is shown by Table I.



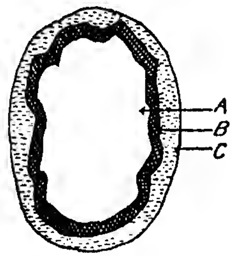


FIG. 1. FIG. 2.
A = Silicate particle
B = Colloid gel, resulting from hydrolysis
C = Water film

TABLE I

ADSORPTION OF CALCIUM ACID PHOSPHATE BY INORGANIC OXIDE GELS

Conc.	Mgm. Ca Ads per gm. Gel			Mgm. PO_4 Ads per gm. Gel		
	Silica	Alumina	Ferric Oxide	Silica	Alumina	Ferric Oxide
N/10	0.12	84.9	121.4	0.08	610.2	609.9
N/20	0.12	51.4	83.3	0.04	393.4	421.6
N/40	0.11	32.7	54.5	0.01	272.6	266.6

The sulphates and nitrates were adsorbed to a much less extent.[4] When the water is moving down through the soil, as after a rain, the soluble salts in the water film diffuse into the moving water. This destroys the salt equilibrium between the incasing gel and water film, and hence, some of the soluble adsorbed salt is released to the water film. The rate at which these soluble salts are removed by this moving column of water may be gleaned from a leaching experiment where the incasing gels have been prepared and allowed to adsorb their maximum quantity of calcium acid phosphate and then leached.

The results of such an experiment are given in Table II.

The leaching was continued until the filtrate failed to give a test for the phosphate unless it was allowed to stand for some time on the gel. About 40,000 cc. had been used at this time. Since the phosphate radical was only slightly absorbed by the silica, it might seem strange that it was so persistent in holding the phosphate. A moment's thought will make this clear. The silica gel has a great capillarity which is filled with this phosphate solution. When water is run over the gel, time is

[4] Lichtenwalner, Flenner and Gordon, *Soil Science*, 15, 157 (1923).

not given for an equilibrium to be established between the leaching water and the solution in the capillaries. This is shown by allowing the water to stand on the gel over night when the phosphate found in a portion of such filtrate is far in excess of that found in a portion of filtrate

TABLE II

QUANTITY OF PHOSPHATE FOUND IN 50 CC. PORTIONS OF FILTRATE

Filtrate Number	PO_4 in 50 cc. of Filtrate from the Hydrogel at the end of 500 cc. Washings		
	Silica	Alumina	Ferric Oxide
	mgm.	mgm.	mgm.
1	4.74	10.0	6.0
2	.14	6.4	3.2
3	.13	2.5	1.5
4	.12	2.1	1.3
5	.12	2.1	1.2
6	.11	1.6	1.0
7	.10	1.2	0.8
8	.09	1.1	0.7
9	.08	0.7	0.7
10	.08	0.4	0.6
Over night	.54	1.2	1.1

which has simply passed over the gel. This is one way nature has provided to protect the soluble salt in times of heavy rains. At the completion of this leaching experiment the gels were analyzed when only a trace of phosphate was found in the silica while the alumina and ferric oxide gels gave the analysis as shown in Table III.

TABLE III[4]

QUANTITY OF PHOSPHATE WASHED FROM GEL

	PO_4 Adsorbed by Hydrogel of Ferric Oxide	PO_4 Adsorbed by Hydrogel of Alumina
	mgm.	mgm.
Before washing	259.0	162.4
After washing	164.0	117.5

In the case of ferric oxide the rain water would apparently remove a little over one-third of the adsorbed phosphate while in the case of the alumina slightly over one-fourth would be removed.

The next question was to find if these salts which were apparently

[4] Lichtenwalner, Flenner and Gordon, *Soil Science*, 15, 157 (1923).

not removed by leaching were available for plant growth. To determine this ferric oxide and aluminum gels which had been leached as above described were thoroughly mixed with pure quartz sand. The gel coated these quartz sand grains in such a way that the particles were very similar to the one given above in Figure II, *i.e.*, a silicate particle incased by an inorganic gel. The pure white quartz sand was placed in 1 *, 5, 2a, 3a, 4a, 6a, 7a, 8a, as shown in Figure III, while the particles incased with ferric oxide gel were placed in 2b, 3b, 4b, and particles incased with alumina were placed in 6b, 7b, 8b. Sweet potato plants were used in the experiment because of the ease with which the roots might be divided between the two adjacent jars. The b jars all received a complete fertilizer solution minus phosphorus; 1a and 2a, 5a and 6a

FIG. 3*.—Sweet potato plants at completion of experiments.

received distilled water, 3a and 7a a complete fertilizer solution minus phosphorus, while 4a and 8a were treated with a complete fertilizer solution. At the end of eight weeks the plants had the appearance as shown in Figure III. Plants 1 and 5 had no source for phosphorus, 2, 3, 6 and 7 had only adsorbed unleachable phosphorus while 4 and 8 had phosphorus in addition to the adsorbed. For details of the experiment and analysis of plants see the original article.[5] The point of the part given is that the roots seem to rap themselves around these soil particles which are incased by these gels containing adsorbed salts and although these adsorbed salts could be leached only in minute quantities yet apparently these inwrapping roots took up the adsorbed salts in sufficient quantities to nourish the plant. This is explained by the fact that we have an equilibrium. When the plant removes this slight amount of soluble phosphorus the equilibrium would be so shifted that more phosphorus would become available. It does not matter

* The numbers 1-8 refer to double jars and 1a and 1b, 2a and 2b, etc., refer to individual jars.

[5] Wiley and Gordon, *Soil Science*, 15, 371 (1923).

whether the plant takes up the phosphate from $CaH_4(PO_4)_2$ or from some complex compound. Only one product of an equilibrium has to be removed from the field of action to carry an equilibrium in that direction and it does not matter whether a plant or some other agent or condition removes said product.

In view of these findings some equilibrium experiments were tried where the alumina and ferric oxide gels were allowed to reach an equilibrium with a 0.1 N solution of the phosphates and then they were diluted to 0.05 N and again brought to equilibrium. These results are given in Table IV.

TABLE IV

EQUILIBRIUM EXPERIMENTS WITH FERRIC OXIDE AND ALUMINA

Conc.	Mgm. of ions Adsorbed per gm. Ferric Oxide Gel						Mgm. of ions Adsorbed per gm of Alumina Gel			
	$MgH_4(PO_4)_2$		$CaH_4(PO_4)_2$		KH_2PO_4		$MgH_4(PO_4)_2$		$CaH_4(PO_4)_2$	
	Mg	PO_4	Ca	PO_4	K	PO_4	Mg	PO_4	Ca	PO_4
N/10	25.0	192.7	57.6	258.7			18.3	172.3	51.6	260.6
Dil to *N*/20	24.0	173.1	51.5	250.6	43.9	165.4	16.5	155.7	48.0	253.3
Orig. *N*/20	21.7	165.0	50.7	235.6	43.8	165.4	14.0	145.0	47.0	239.0

The process did not seem to be completely reversible but the equilibrium had a tendency to apply only to that part which was leachable.

The adsorption of the sulfates and nitrates were completely reversible when run similar to the phosphates.

Since the phosphates were more highly adsorbed than the sulfates, they should be able to displace any adsorbed sulfates. Table V shows how complete such replacement is.

TABLE V*

REPLACEMENT OF ADSORBED SO_4 BY PO_4 IN GELS

	Mgm of ion Adsorbed per gm. Alumina Hydrogel						Mgm of ion Adsorbed per gm. of Ferric Oxide Hydrogel					
	K_2SO_4		$MgSO_4$		$CaSO_4$		K_2SO_4		$MgSO_4$		$CaSO_4$	
	K	SO_4	Mg	SO_4	Ca	SO_4	K	SO_4	Mg	SO_4	Ca	SO_4
Adsorption before adding $MgH_4(PO_4)_2$	11.0	19.0	6.2	26.3	5.7	12.0	11.4	13.6	7.6	21.1	12.9	36.0
Adsorption after adding $MgH_4(PO_4)_2$	16.1	—	11.1	—	13.1	—	29.0	—	25.2	—	24.0	—

* Lichtenwalner, Flenner, Gordon, 15, 158 (1923).

Up to this point the discussion has emphasized the adsorption of the negative ions. We will now give more attention to the positive ions. Silica has been most carefully investigated from this standpoint in our laboratory. A few of the results will be given.

We believe that much of the metallic adsorption by soil colloids was due to the silica, and furthermore, that some of the acidity of the soil and metal displacements found in soil work might be attributed to colloidal silica.

To investigate these points, the silica was first allowed to adsorb or react with sodium hydroxide until a 0.02 N solution placed on the gel gave a neutral reaction, then the silica was placed in contact with 0.02 N solution of sodium nitrate and shaken until equilibrium was established. Similar experiments were run where potassium, magnesium, silver and calcium nitrates and nitric acid were used in place of the sodium nitrate. A second series of experiments were run where a 0.04 N solution of the nitrates and nitric acid were used in place of the 0.02 N solution. Table VI gives the adsorption found in the respective experiments.

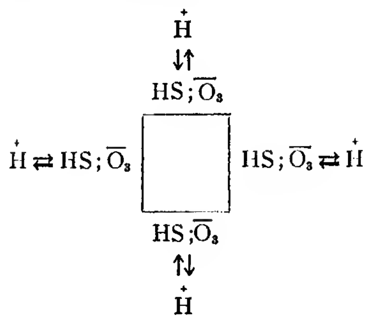
TABLE VI[7]

ADSORPTION OF METALS BY HYDROGEL OF SILICA

Solutions Used		Equivalents of Na Ads per gm. Gel	Equivalents of M[8] Ads per gm. Gel	Total Equivalent of Metals Ads per gm. of Gel
$C = 0.02\,N$	$C = 0.02\,N$			
$Na(OH)$		36×10^{-5}		36×10^{-5}
$Na(OH)$	$Na(NO_3)$	45×10^{-5}		45×10^{-5}
$Na(OH)$	$K(NO_3)$	14×10^{-5}	32×10^{-5}	45×10^{-5}
$Na(OH)$	$Mg(NO_3)_2$	13×10^{-5}	32×10^{-5}	45×10^{-5}
$Na(OH)$	$Ag(NO_3)$	12×10^{-5}	33×10^{-5}	45×10^{-5}
$Na(OH)$	$Ca(NO_3)_2$	-2×10^{-5}	38×10^{-5}	36×10^{-5}
$Na(OH)$	$H(NO_3)$	-2×10^{-5}	48×10^{-5}	46×10^{-5}
$C = 0.02\,N$	$C = 0.04\,N$			
$Na(OH)$	$Na(NO_3)$	47×10^{-5}		47×10^{-5}
$Na(OH)$	$K(NO_3)$	7×10^{-5}	39×10^{-5}	46×10^{-5}
$Na(OH)$	$Mg(NO_3)_2$	7×10^{-5}	40×10^{-5}	47×10^{-5}
$Na(OH)$	$Ag(NO_3)$	5×10^{-5}	42×10^{-5}	47×10^{-5}
$Na(OH)$	$Ca(NO_3)_2$	-4×10^{-5}	44×10^{-5}	40×10^{-5}
$Na(OH)$	$H(NO_3)$	-4×10^{-5}	44×10^{-5}	39×10^{-5}

These experimental facts lead us to believe that a certain number of the surface molecules of the silica ultramicrons were ionized, and that the ionization might be represented thus:[9]

[7] This table is taken from E. B. Starkey's dissertation for Doctor's Degree at the University of Maryland. Not yet published.
[8] M = Metal other than sodium.
[9] Gordon, *Science*, 58, 495 (1923).



When the sodium hydroxide was placed on the silica we have a simple case of neutralization which might be expressed as follows:

$$\square\, H\overline{SiO_3} + \overset{+}{H} + \overline{HO} + \overset{+}{Na} \longrightarrow \square\, H\overline{SiO_3} + \overset{+}{Na} + H_2O \qquad (1)$$

When this is treated with a potassium salt, as potassium nitrate, the potassium would replace the sodium, if the potassium silicate is less soluble than the sodium silicate, and in turn the silver would replace the sodium to a greater extent than did the potassium, provided the silver silicate is less soluble than the potassium silicate, *i.e.*, we should have the equations

$$\square H\overline{SiO_3} + \overset{+}{Na} + \overset{+}{K} + \overline{NO_3} \rightleftarrows \square H\overline{SiO_3} + \overset{+}{K} + \overset{+}{Na} + \overline{NO_3} \qquad (2)$$

and

$$\square H\overline{SiO_3} + \overset{+}{Na} + \overset{+}{Ag} + \overline{NO_3} \rightleftarrows \square H\overline{SiO_3} + \overset{+}{Ag} + \overset{+}{Na} + \overline{NO_3} \qquad (3)$$

Where equation (3) should go nearer completion than equation (2). Results given in Table VI show this to be the case. The results given in Table VI also indicate that the hydrogen and calcium ions have the greatest tendency to displace the sodium. This is simply because the silicates which they form are the most insoluble of any tried.

Charge on Silica Gel

The interpretation given above offers an explanation for the charge on the silica gel. All know that silica is negative with respect to water. A glance at figure III clearly shows that the ultramicron with its surface ionization must have a negative charge. Furthermore, the charge on this ultramicron should be much larger than it is on a single ion, because each ultramicron would undoubtedly have many surface molecules

ionized. Both these facts are known to be true. Again one would expect that increased acidity should decrease the negative charge on the gel by holding back the surface ionization. This is again true to facts as is shown by Table VIII taken from some of our work [10] on electro-endosmosis where silica gel was used as the membrane. The work was carried out in apparatus similar to that used by Briggs.[11]

TABLE VIII

EFFECT OF PH ON THE ELECTRICAL CHARGE ON SILICA GEL

PH Values	Charge on Gel	Rate of Travel of Water in mm. per sec.	E M F
6.526	Neg	6.3	116
4.717	Neg	3.1	120
3.567	Neg	2.4	120
1.217	Pos	1.4	119

As the acidity increased the ionization of these surface molecules should decrease, but one would not expect to get an entire depression of the ionization until the acidity had become very great. The charge was still negative at a PH of 3.567, but when the PH reached 1.217, the gel had become positive. This last fact might be explained by the surface molecules beginning to function basic in this great acidity. In other words, silica gel can be made to function amphoterically, provided the acidity is sufficiently great.

$$\underbrace{\overset{+}{H} + HS\overline{i}O_3}_{\xleftarrow{\quad}\ PH > 1.217} \rightleftarrows H_2SiO_3 \rightleftarrows \underbrace{H\overset{+}{Si}O_2 + \overset{-}{O}H}_{\xrightarrow{\quad}\ PH < 1.217}$$

When the PH is greater than 1.217 the silica functions as an acid, while it functions as a base when the PH has become less than 1.217.

This interpretation of the structure of silica gel helps to explain phenomena connected with soils, such as peptization and flocculation, replacement, acidity, etc.

PEPTIZATION AND FLOCCULATION

When the reaction shown in equation (1) takes place, we have the sodium ions in equilibrium with the silicate ions at the surface of the ultramicron. These sodium ions would have a tendency to diffuse through the solution, but they could not go without taking the ultramicron in their train. This would happen if the ultramicron did not have too great mass, and in such a case, we would have peptization,

[10] Taken from James Elder's unpublished work.
[11] Briggs, *J. Phy. Chem.*, 21, 198 (1917).

but if the mass of the ultramicron is too great, we will have the sodium held in the vicinity of the ultramicron and so-called adsorption results. Experimental facts gave such results. When the silica gel was treated with sodium hydroxide until the alkalinity had disappeared and the analysis run, it was found that part of the sodium was found in the solution with its accompanying ultramicron which had a mass too great for migration. The surface of the unmigratable ultramicron is so great that a fairly high so-called adsorption resulted. This maximum change of adsorption and resulting peptization takes place around a PH of 7.

This curve was published by Gordon and Starkey in the Soil Science.[12] The paper was read before the New York Meeting of the A. C. S. in the fall of 1920 and the theory was given before the Boston meeting of the American Association for the Advancement of Science in the fall of 1922.[13]

It is very interesting to note that Bradfield [14] has recently obtained a similar curve for peptization where he has used the soil colloid as a whole. He has assumed a complex where his silica is functioning in the complex similar to the way we have assumed the silica to function in an isolated form. If such complexes did exist, would we find such a variation in the composition of soil colloids? Dr. Bradfield [15] advances the argument that mixtures of artificial colloids, when made up of the same composition as colloids found in soil, do not possess the same properties as soil colloids. Would you expect this since the natural soil colloids have already been subjected to soil salts ageing and other soil conditions?

Acidity of the Soil

The assumptions made for silica given in Fig. 3 would also help to explain the acidity of the soil. No special emphasis was placed on this when first presenting the work, for it was supposed that it was self-evident from what was said that a certain amount of the soil acidity must result from such a surface ionization, but the author has no reason to believe that the ionization of the colloids accounts for all the acidity of the soil. It would seem that the acidity of the soil results from one great chemical equilibrium of the soil salts where hydrolysis is the principal factor. Whether you have an acid or basic soil depends upon the state of hydrolysis and the salts present. The soil colloids resulting from hydrolysis are only one of the contributing factors. For example, we express the hydrolysis of ferric chloride as follows:

[12] Gordon and Starkey, *Soil Science*, 14, 1 (1922).
[13] Gordon, *Science*, 58, 495 (1923).
[14] Bradfield. Colloidal Symposium (monograph), p. 377, 1928, Univ. of Wis.
[15] *Ibid.*, p. 886, 1923, Univ. of Wis.

$$FeCl_3 \rightleftarrows \overset{+++}{Fe} + 3\bar{Cl}$$
$$3H_2O \rightleftarrows 3\bar{OH} + 3\overset{+}{H}$$
$$\downarrow \qquad \uparrow\downarrow$$
$$Fe(OH_3) \quad 3HCl$$

Where it is evident that the colloidal ferric hydroxide plays a small rôle directly in the acidity of the soil in comparison with the other product of hydrolysis, namely, HCl. As soon as the HCl is formed it may immediately react with something else and in any case an acid equilibrium results.

Potassium Content of Soil Increased by Lime or Acid

As far as colloidal silica is concerned, potassium is released either by a base or an acid. If silica were the only holding power of the soil, it would be possible to always increase the potassium content of the soil by the use of lime. Table VI bears out this statement, but soil conditions prevent this being true except in some cases. On the other hand researches such as that of A. G. McCall and A. M. Smith [16] are cases where potassium has been released by increasing the acidity. They added sulfur to different green sand marls and varied conditions so that varying amounts of sulfur were oxidized over to sulfur acids. Where the greatest amount of sulfur was oxidized the largest quantity of water soluble potassium was produced. Here the system was less complex than in ordinary soil and hence Table VI lends itself to the proper interpretation of the experiment. If the outer layer of the silicate particle had undergone decomposition, as shown in Figure II, the sand grain would be largely incased by colloidal silica. From Table VI it is clear that the more acid the less the potassium is adsorbed or the more potassium might be leached. This is what was found.

Adsorption of Dyes by Soil Colloids

Since dyes had been used to some extent to determine the colloidal content of the soil, and since the acid had such an effect on the adsorption, it became of interest to see how the PH affected the adsorption of dyes by soil colloids. Table IX shows the adsorption of Orange II by the three principal colloids of the soil.

[16] McCall and Smith, *Jour. Agr. Res.*, 19, 239 (1920).

TABLE IX[11]

ADSORPTION OF ORANGE II BY SOIL COLLOIDS

Silica Hydrogel		Ferric Oxide Hydrogel		Alumina Hydrogel	
PH	Ads per gm. Gel	PH	Ads per gm. Gel	PH	Ads per gm. Gel
2.3	0.00	2.3	429.0	2.30	452
3.2	0.00	3.2	78.0	3.20	196
5.37	0.00	5.37	70.0	5.37	179
10.14	0.00	10.14	52.0	10.14	162
11.02	0.00	11.02	50.0	11.02	136

This table is best illustrated by the following graph:

The very sharp change in adsorption at the low PH(2.3) prompted us to carry on an investigation to find the exact cause of the sudden

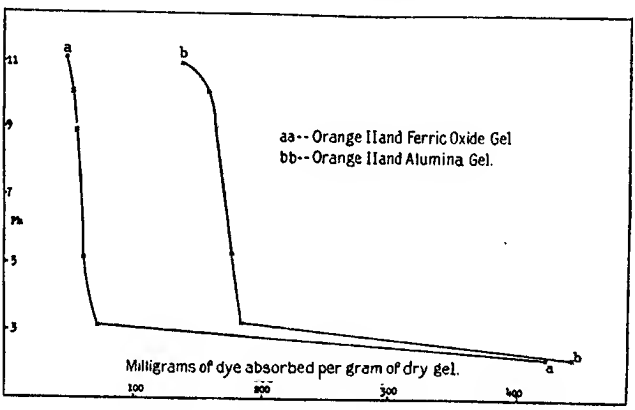


FIG. 4.

break. Of course, the first suggestion was that a compound was formed at this particular PH. Our work has established the fact that a true chemical equilibrium exists where certain dyes are used with the soil colloids. In the case of Orange II we have:

$$3XH + Al(OH_3) \rightleftarrows AlX_3 + 3H_2O$$
$$\text{where } X = C_6H_5.N = NC_{10}H_6(OH)$$

This shows that when certain dyes are used to determine the quantity of colloids in the soil the adsorption is a true chemical reaction. Since the completion of this chemical reaction depends on the acidity, it is

[11] Marker and Gordon, *J. Eng. Chem.*, 16, 1186 (1924).

impossible to get a true estimate of the colloidal content of soils by dye adsorption.

Summary

In this paper the following points have been developed:

1. The origin of soil colloids
2. The adsorption of fertilizer salts by the individual soil colloids
3. The rate at which salts are leached from the soil
4. The power of plants to utilize adsorbed salts
5. Adsorption as an equilibrium
6. Replacements of both negative and positive radicals in soil colloids, where such replacements help to explain (*a*) charge on soil colloids, (*b*) Metallic and non-metallic replacements in soils, (*c*) peptization or flocculation of soil colloids, and (*d*) acidity of soils
7. Soil acidity results from a chemical equilibrium. The soil colloids being an insoluble product in this equilibrium, cause the hydrolysis to take place to a large extent, but in themselves furnish only a slight amount of the acidity in comparison with the other hydrolytic products formed simultaneously with the soil colloid
8. Dyes cannot be used to ascertain the colloidal content of soils.

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THE RÔLE OF COLLOIDS IN SOIL MOISTURE

George John Bouyoucos

Introduction

In the earlier history of soil investigation when the importance of colloids was not recognized or taken into account, the relationships existing between soil and water were considered to be simple, and were interpreted on the hypothesis that the soil is a static framework upon which the liquid plays its rôle. This conception is well reflected by the attempts that have been made to measure the movement and average thickness of the moisture films, to calculate the specific surface of the soil from the size of its particles, and using sand grains, marbles or small bullets, for many such studies.

With our present recognition of the colloidal and absorptive properties of the soil, however, we can no longer regard the soil as a simple, inactive mass of particles with no influence of its own upon the water, or the water upon it. It is now generally admitted that colloidal material does exist in the soil derived both from the organic and inorganic constituents and it may exist primarily as a coating around the soil grains, and to a smaller degree as an independent component scattered throughout the soil grains. It now appears that this colloidal material is probably responsible for most, if not all, of the activities of the soil, both chemical and physical, exerts a controlling influence on the water relationships and makes these relationships between soil and water most intimate and complex. To fully recognize and appreciate the great and controlling influence, or the important rôle that colloids play in soil moisture, it is necessary to direct attention to, and examine, some of the most important water relationships and moisture phenomena in soils. These water relationships and moisture phenomena may be grouped under the general headings of attraction, adhesion, heat of wetting, freezing point depression, unfree water, vapor pressure, evaporation, wilting coefficient, water holding capacity, capillary movement, percolation, etc. The influence of colloids upon each of these properties or phenomena will be considered separately but briefly.

Attraction

All soil material possesses an attractive power for water, but the magnitude of this attractive power varies with the colloidal content

of soils. This attractive power of soils for water is well manifested and illustrated in their hygroscopic water content. When dry soils are placed in a saturated atmosphere they will absorb water vapor until a condition of approximate equilibrium is attained. The amount of hygroscopic water that soils of various colloidal contents may absorb is shown in Table I. The values have been obtained by Briggs and Shantz.[1]

TABLE I

HYGROSCOPIC COEFFICIENT OF SOILS

Type of Soil	Hygroscopic Coefficient Per cent.
Coarse sand	0.5
Fine sand	1.5
Sandy loam	2.3
Fine sandy loam	6.5
Loam	9.8
Clay loam	11.8
Clay	13.2
Clay	14.6

It is readily seen that the finer the soil, the greater is the hygroscopicity. The finer the soil the greater is also the percentage of clay, and consequently the greater is the amount of material likely to be in a colloidal state. As a matter of fact, the hygroscopic moisture tends to be roughly proportional to the clay; and as clay, especially the finer forms, is largely colloidal in nature, the colloidal content of soil practically determines the hygroscopic content. It is this fact upon which Mitscherlich [2] bases his method of colloidal estimation in which hygroscopic moisture determined under certain controlled condition, is used as a relative measure of colloidal content. The U. S. Bureau of Soils [3] has also recently made use of the same principle, namely, the attraction or adsorption of water vapor by soils to estimate their colloidal content. The results that the investigators of this Bureau obtained show that more than 95 per cent of the adsorbtive capacity of a soil is due to its colloidal material and only about 5 per cent to its non-colloidal material.

HEAT OF WETTING

The attractive power of soils for water and the influence of colloids on it, is also shown by the phenomenon of heat of wetting. The work of Müntz and Gandechon,[4] and of Bouyoucos [5] shows that the amount

[1] U. S. Dept. of Agr., Bur. Plant Industry, Bulletin 230 (1912).
[2] *Internat. Mitt f. Bodenkunde.* Band 1. Heft 5. Seite, 463-480 (1912).
[3] U. S. Dept. Agr., Bull. 1193 (1924).
[4] *Ann. Sci. Agron.* (3 sir), 4, 11 (1909), p. 393.
[5] Mich. Agr. Expt. Sta., Tech. Bull. No. 42 (1918), p. 8.

of heat that dry soils will evolve upon wetting increases with the fineness of particles or colloidal content as shown in Table II.

TABLE II

Heat Evolved on Wetting Dry Soils (Bouyoucos)

Soil Type	Heat evolved by 50 grams of Soil—Calories
Quartz sand	0.0
Coarse sand	0.2
Fine sand	0.8
Sandy loam	10.8
Fine sandy loam	15.0
Loam	172.8
Silt loam	205.2
Clay loam	391.5
Clay	607.5

Bouyoucos[6] also found that on heating these soils to various temperatures their heat of wetting was gradually decreased until a temperature of about 750° C. was reached at which the heat of wetting disappeared entirely, in all soils. After these soils were heated to the above temperature they would yield no heat of wetting irrespective of how fine they were ground again, indicating that ignition destroyed their attraction for water. The attraction of colloids upon water does not always depend upon the size of particles, but more particularly upon their nature and state of activation. As stated above clay soils in their natural state give a tremendous amount of heat of wetting but after they are burned they refuse to produce any heat of wetting no matter how fine they are reduced in size again. The state of activation of colloids may depend upon their degree of decomposition and upon the nature of surface, *i.e.*, whether it is porous, smooth, vitrified, etc.

The phenomenon of heat of wetting has been used and proposed by Bouyoucos as a means of estimating the colloidal content of soils. The procedure consists of extracting some of the colloids of the soils and determining their heat of wetting. The percentage of colloids in the soil is then calculated by the ratio

$$\frac{\text{heat of wetting per gram of soil}}{\text{heat of wetting per gram of colloid}} \times 100.$$

The method appears to be able to give a fairly reliable estimation of the active colloids in the soil. According to this method, the colloidal content of soils ranges from 0 to as high as 80 per cent. The results go to show that the phenomenon of heat of wetting is due almost entirely to the colloids, as soils with high heat of wetting cease to possess this property when deprived or washed free from colloids. Thus,

[6] *Soil Science*, Vol. 17, No. 2. Feb., 1924.

for instance, a soil which originally had a heat of wetting of 17.750 calories per gram, had only 5.248 calories after freed of some of its colloids.

The colloids of the different soils give a much different heat of wetting per gram of material. The difference found ranges from 8.520 to 18.956 calories. The power of the colloids from different soils to give different heat of wetting is attributed to their difference in nature and to their difference in activity. The latter represents probably difference in the degree of decomposition, and difference in nature of surface: whether it is porous, smooth, vitrified, etc.

Adhesion

The water which the soils attract or absorb is held with a certain amount of adhesive forces. The magnitude of these adhesive forces decrease with the increase of moisture content but at very low water content they are very great, especially in soils with high colloidal content. No method has yet succeeded in measuring this adhesive force with any degree of precision but the researches of Lagergren,[7] Young,[8] and Lord Rayleigh[9] indicate that it might have an order of magnitude of from 600 to 25,000 atmospheres. More recently Shull[10] attempted to measure the adhesive forces of soils for water by determining the moisture content at which zanthium seed could no longer take up water from the soil, and the points of equilibrium between the seed and soil at different moisture contents. These results were compared with similar data in which the seed was placed in salt solutions of known osmotic pressure. The seed reduced the moisture content of the soil to an air dry condition, and at this point the soil held the water with a force equal to about 1000 atmospheres. A clay soil, however, which indicates this adhesive force still contained 6.66 per cent of moisture while a sand contained only 0.159 per cent.

Briggs and McLane[11] in attempting to separate the water from the soil by whirling wetted soils in a rapidly revolving centrifuge, filled with a filtering device in the periphery, and developing a force equivalent on the average of 3,000 times the attraction of gravity, found that some clay soils would still contain about 46 per cent of water, while sand with practically no colloidal material would contain only 3 per cent.

Wilting Coefficient

The great adhesive forces that soils exert for water and its difference in magnitude in the colloidal and non-colloidal soils, is further illustrated in the wilting coefficient studies of plants. Briggs[12] and Shantz, and

[7] *Behand. till K. Sv. Ve-tokakt.* Hand. 24, afd., 11, 5 (1898).
[8] "Hydrostatics and elementary hydrokinetics," G. M. Minchin, p. 311 (1692).
[9] *Phil. Mag.*, 5, 30, 285-298, 456-475 (1890).
[10] *Bot. Gaz. v.*, 62, No. 1, p. 1-31 (1916).
[11] U. S. Dept. Agr., Bureau of Soils, Bull. No. 45 (1907).
[12] U. S. Dept. Agr., Bureau of Plant Industry, Bull. 230 (1912).

others, have shown that plants wilt and die in some clay soils when they still contain as much as 20 per cent of water, while in the case of some sands, practically devoid of colloids, the plants reduce the moisture content to less than one per cent before they begin to wilt and die.

Evaporation

According to the investigations of Cameron and Gallagher,[13] and especially those of Keen,[14] the evaporation of soil moisture at the lower water contents is controlled mainly by the colloids. Keen found that the rate of evaporation in clay at low moisture contents is very much different in character than that in sand. In the case of sand, and even in silt, China clay, and ignited soil, the evaporation was simple in character and could be explained by the known laws of evaporation and diffusion. But in the case of colloidal clays it was more complex and could not be accounted for by the same laws. Instead of a simple proportionality between water content and rate of evaporation found in the case of sand, the curves for the colloidal soils were more of exponential in type. The difference was due to the colloids as shown by the fact that it disappeared when the soils were ignited and their colloidal properties destroyed. After ignition the curves of evaporation for the colloidal soils became the same as in sand, in spite of the fact that the ignited soil still contained a very large number of very small particles.

Vapor Pressure

These results on the rate of evaporation indicate that colloids must affect the vapor pressure, and indeed such appears to be the case. Thomas[15] found, for instance, that the vapor pressure of sand with a moisture content of 0.70 per cent was 22.737 mm. while a clay loam with 2.65 per cent of moisture gave a vapor pressure as low as 10.65 mm. While the difference in the vapor pressure in these two soils is partly due to the difference in the curvature of the water edges between the soil particles due to the surface tension of the liquid, it is due to a greater extent also to the differences of the attractive forces of the two soils for water.

Freezing Point Depression

Another remarkable phenomenon of colloids towards water is their effect on its freezing point depression. Bouyoucos and McCool[16] have discovered that soils cause a lowering of the freezing point and the magnitude of this value varies with their colloidal content. By

[13] U. S. Dept. Agr., Bureau of Soils, Bull. 50 (1908).
[14] *Jour. Agr. Sci.*, 6, No. 4 (1914).
[15] *Soil Science*, 11, No. 6 (1921).
[16] Mich. Agr. Expt. Sta. Tech. Bull. 31 ('916).

comparing the freezing point depression of non-colloidal soils with that of the heavy colloidal types at different moisture contents, many significant differences are discovered. In the first place, at the lowest moisture content at which it is possible to make a freezing point determination, quartz sand and coarse sandy soils at a moisture content of only 1 per cent gave a freezing point depression of only 0.090° C., while many clay soils at as high moisture content as 20 per cent gave a freezing point depression of 1.500° C. These values were obtained after the soils were washed free from their soluble material. In the second place, the freezing point depression values at the different moisture contents show that in the case of the sands they tend to increase inversely proportionally to the moisture content as it should be expected, while in the case of the colloidal soils, they tend to increase in a geometric progression as the moisture content decreases in an arithmetic progression. Evidently, therefore, soil colloids have an abnormal and greater influence on the freezing point depression of the soil water films than the non-colloidal soils.

Although a part of the abnormally great depression of the colloidal soils at the lowest moisture content is due to the soluble material held adsorbed it appears very probable, Parker,[17] that a considerable part is due also to physical factors. In other words, the soil particles or colloids exert a physical effect upon the freezing point at the lowest moisture content. These physical factors appear to be (1) the attraction of the soil for water and (2) the thinness of the water films around and between the soil particles. In just what manner or what is the mechanism by which these two factors affect the freezing point is not definitely known. The effect, however, seems to be more like that of the dissolved materials or ions than of the mechanical pressure.

This abnormally high freezing point depression of the colloidal soils at the lower moisture content is of considerable importance in the water relationships of plants. At low moisture contents the plants are unable to utilize the moisture present and they wilt, even though there still may be considerable moisture present. In the light of the abnormally high freezing point depression at the wilting coefficient, the explanation appears evident, namely, that the osmotic pressure at the wilting coefficient is very high, higher than the plant cell sap and consequently the plants cannot extract water from the soil. It must be emphasized that whether the freezing point depression is caused by soluble material or by the physical factors, the result on the osmotic pressure and consequently on the plant, is the same.

Unfree Water

Coincident with the property of soils or colloids to cause a lowering of the freezing point, is the amount of water that fails to freeze on

[17] *Journ. Amer. Chem. Soc.*, 43, p. 1011 (1921)

same. Bouyoucos[18] found that when soils are caused to freeze some of their water fails to freeze and the amount of this unfrozen water varies with the colloidal content present. Foote and Santon[19] working with hydrogels obtained similar results. The determination of the unfrozen water in soils was made by means of the dilatometer method. The results yielded by this method show that when soils are supercooled to — 1° or to — 4° C. then frozen at — 78° C., and then brought back to their original cooling temperature large amounts of water remain unfrozen, especially in the colloidal types of soils. In some of the colloidal clays for instance, the amount of water that fails to freeze at the temperature of — 4° C. after they were frozen at — 78° C. is about 20 per cent, and in colloidal mucks about 60 per cent, while in sandy soils it is generally less than 1 per cent. If the soils are frozen at only — 1° C., the amount of water that fails to freeze is much greater in the colloidal soils than the amounts shown above. The water which fails to freeze near zero has been designated by Bouyoucos as unfree water in contrast to free water which readily freezes near the zero temperature. The free water is beyond the influences of the soil particles and freezes as liquid in mass, while the unfree water is under the influences of the soil particles and is not free to freeze like liquid in mass.

Just what is the actual condition of the unfree water, cannot be definitely stated at present. It probably exists, however, mostly as capillary adsorbed water.

Moisture Holding Capacity

The rôle of colloids is also manifested in the total water holding capacity of soils. Although this property is influenced to considerable extent by the height of the soil column and by the mode of packing of the soil particles, it is probably influenced to a still greater extent by the colloids and by the amount of organic matter which may be classed as colloids and by the size of soil particles which, if fine enough, may be also classed as colloids. The colloids and organic matter besides having an important bearing on the formation of compound particles, and hence directly on the pore space, also imbibe large quantities of water and swell considerably thus increasing the effective volume of a given weight of soil and allowing it to take up more water than it would otherwise do. The difference in the moisture holding capacity between colloidal and non-colloidal soils is very marked. In unpublished results Bouyoucos found that some ordinary clays will hold as much as 75 per cent of water as compared to only 20 per cent in some

[18] Mich. Agr. Expt. Sta., Tech. Bull. 86 (1917).
[19] *Jour. Amer. Chem. Soc.*, Vol. 38, No. 3, pp. 588-609 (1916).

coarse sands. Similar results have been obtained by King,[20] Alway and McCole [21] and others.

Permeability

The property of colloids to swell upon imbibition of water and to deflocculate under certain conditions, play a very important rôle in the permeability of soils to water. Water percolates with considerable difficulty in soils with high colloidal content even under field conditions. When these colloidal soils are dry and only slightly compacted, the swelling of the gel portion of the colloids tends to close the pores in the soil thus stopping up the channels of percolation and making the soil practically impermeable. This is especially true at higher temperatures as shown by Bouyoucos.[22] The deflocculation of colloids brought about either by the leaching out of certain salts which tended to keep them in the flocculent condition or by the presence or addition of salts which have a deflocculating effect, have also the same and even greater effect on the permeability of soils to water as the swelling of the gels have. This latter effect is well illustrated by the work of Sharp.[23] On account of these effects of the colloids it is futile to attempt to express the rate of flow of water in soils quantitatively according to the formula of Poiseuille.

Capillary Rise

What is true of the effect of colloids on the percolation of water is also true on the capillary rise of water in soils. Water tends to rise at a much slower rate in a column of dry clay than it does in coarse sand. Undoubtedly the principal cause for this slower rate of capillary movement in clay is due to the swelling of the colloidal fraction which tends to constrict or close up the capillary pores, make the whole soil mass more tight and thereby create a greater friction to the passage of water. Experimental evidences seem to support this explanation. If columns of dry clay are placed over water, ligroin, ether, benzol, toluol and kerosene it is found that these organic liquids rise in the soil at the beginning four or five times faster than does water, and at the end of three days they have travelled four or five times higher than has water; and they have done this in spite of the fact that their surface tension is much below that of water. On the other hand water rose faster in fine quartz sand than did the organic liquids. The apparent explanation for these phenomena is that the organic liquids wet the soil but they are not imbibed to cause a swelling of the gel and consequently they travel the pore channels unhindered. The clay has a

[20] Wis. Agr. Expt. Sta., 6th Rept. (1889), p. 189.
[21] *Jour. Agr. Res.*, 14, p. 27 (1917).
[22] Mich. Agr. Expt. Sta., Tech. Bull. 22 (1916).
[23] Univ. Calif. Pubs. Agr. Sci., 1 (1916), No. 10, pp. 291-339.

sufficient attraction for them, and since it is very fine and has a large number of interstitial angles it is able to support a high column of their liquid. In the case of fine quartz sand, however, the water with its higher surface tension and open and unhindered capillary channels, rose at a greater rate than the organic liquids with the low surface tension.

Although on account of the greater friction due to the swelling of the colloidal gels and to other factors, the water will rise at a slower rate in the colloidal than in the non-colloidal soil, it will finally rise to a much greater height in the former than in the latter. This is due partly to the greater attraction and absorption that colloids have for water, and partly to the ability of the fine particles and colloids to support a higher column of liquid due to their greater number of interstitial angles.

Conclusions

Although it appears evident from the facts presented that colloids exert a most important, if not the controlling influence, on the water relationship of soils, it must be strongly emphasized, however, that our knowledge of these various relationships as influenced by colloids, is far from being complete or absolutely definite. Indeed, all the subjects here considered need further, deeper, and more comprehensive investigation. The information we possess at present on most of these subjects is more of a qualitative nature, and should be reduced, if possible, to a quantitative basis. More definite explanations are also needed for the results of many of the subjects. The rôle of colloids of soil moisture, therefore, presents many problems in need of solution, and excellent opportunities for investigation.

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POLAR EMULSIFYING AGENTS

By Harry N. Holmes and H. A. Williams

The conception of definite orientation of molecules at surfaces of liquids was first offered by W. B. Hardy in 1912. Langmuir [1] later suggested that in a layer of fatty acid floating on water the —COOH groups strike into the water. Harkins, Davies and Clark [2] believed that at the interface between water and a liquid hydrocarbon molecules of an added polar substance (such as R.COOH) would tend to orient themselves so as to throw the —COOH (or other polar group) into the water and the non-polar hydrocarbon radical into the non-polar benzene. Harkins and his associates were convinced that the best emulsifying agents were made up of long polar molecules.

Hildebrand [3] prepared an emulsion of benzene in water using oleic acid as the emulsifying agent as did Whitby. No particular experimental study, however, was made of emulsifying agents in *true solution* in the liquids emulsified. In fact the general impression has been that emulsifying agents must be in colloidal dispersion with the exception of the fine-grained precipitates used by Pickering. Briggs [4] stated that "In every case investigation has shown that the apparently soluble emulsifier is in colloidal suspension in the outside phase of the emulsion." Following Gibbs' theory that any substance lowering surface tension must tend to concentrate at the surface it has been held that this would account for interfacial emulsion films. Bancroft,[5] as Clayton says, "extended the modern adsorption film theory and pointed out that, for a substance to act as an emulsifying agent, it must be adsorbed into the interface between the two liquids and form a coherent film there."

We decided to investigate the classes of polar emulsifying agents not commonly supposed to be colloidally aggregated in the liquids used.

The Alcohols.—Benzene was emulsified in water by the use of methyl or ethyl alcohol as emulsifying agent. Propyl alcohol was inferior and the butyl alcohols poor. In fact the higher alcohols were useless as emulsifying agents. Glycol, glycerol and the sugars had no noticeable value yet allyl alcohol showed a pronounced effect, not equal, however, to that of ethyl alcohol. Phenol and resorcinol showed distinct power to emulsify benzene in water but the naphthols did not. Nor did benzyl alcohol.

We found it advisable to clean the bottles with unusual care. After

dissolving 2 cc. of methyl alcohol in 10 cc. of water we could add in small portions, with vigorous hand shaking, 10 cc. of benzene and secure a very good emulsion stable for a week or more. Apparently the CH_3 group of the alcohol is attracted and pulled into the non-polar benzene while the —OH group is pulled into the polar water. Of course alcohol dissolves in both liquids but at the interface a monomolecular layer of alcohol must be held by the opposing attractions in such a way as to form a real film. In the lower alcohols the balance between the CH_3— or C_2H_5— and —OH radicals must be such as to give the optimum effect.

The Acids.—Since it was already known that fatty acids could act as emulsifying agents it remained only to learn just where in the fatty acid series this property begins. Stearic, palmitic, oleic, linoleic and linolenic acids were found to be very efficient but acids below caprylic in the series were of no use. This parallels the emulsifying properties of soaps of this same series. The sulfonic acids, such as B-naphthylamine sulfonic, showed distinct but not unusual emulsifying power. It is interesting here to note that the sodium salt of the latter acid is far superior to the free acid.

Since oleic acid, for example, is insoluble in water it is evident that an interfacial film must form as a result of the —COOH groups sticking into the polar water. The hydrocarbon chain is attracted by the non-polar benzene.

The Esters, Aldehydes, Ketones and Nitriles.—Neutral fats showed noticeable ability to act as emulsifying agents for the benzene-water system but they were quite inferior to rancid fats. We have on our shelves an emulsion of benzene-in-water prepared four months ago, using a very rancid fat as emulsifying agent. Of course the —COOR group of an ester with a hydrocarbon radical introduced must be less polar than the —COOH group of a free fatty acid.

The aldehydes showed very little value as aids to emulsification but benzo-nitril compared rather well with some of the best polar agents. Nitro and amino compounds had little if any effect nor did the ketones.

Interfacial Tension.—Oleic acid does not lower the surface tension (against air) of benzene but it does sharply lower the interfacial tension of benzene-water. The formation of a film in this instance would follow the Gibbs' theory. Whatever its cause the lowering of interfacial tension aids emulsification as pointed out by Hillyer,[6] Donnan[7] and others. Of course alcohol, soluble in both benzene and water, might be expected to produce great lowering of interfacial tension and it does but oleic acid is insoluble in water.

And yet we must be careful not to assume that any substance soluble in both benzene and water and causing at least a distinct lowering of interfacial tension will act as a good emulsifying agent. Acetone is soluble in both of these liquids and shows no emulsifying power.

Nor does methyl-ethyl ketone. We need the pull from both liquids to hold more or less firmly an interfacial film of a polar substance. Acetone is not very polar while methyl alcohol is.

Molecular Association.—It has been objected that our "polar" emulsifying agents might not really be in true solution but might be highly polymerized in one of the liquids. It is quite true that ethyl alcohol, for instance, is somewhat associated in such non-polar solvents as benzene but the freezing point lowering in solutions of the concentration used indicated molecular weights of less than 200 hence the aggregates contain only four or five molecules, very far from colloidal dimensions. In water the alcohol is not associated at all. Even the higher fatty acids in benzene show a rather small degree of association. It is difficult to explain film formation as due to such small aggregates when sugar with a molecular weight of 342 in water has almost no emulsifying power.

Furthermore if the effect in film formation is due to aggregation of alcohol in the benzene, water should be the dispersed phase according to the well-known rule. In reality it is the benzene that is dispersed in drops.

Finally we may cite the fact that addition of alcohol makes it possible to emulsify Nujol in water yet alcohol is not soluble in Nujol and is in true solution in water. This apparently forces us to the polar explanation.

Further Observations.—Gum dammar shows remarkable power to emulsify water-in-oil and yet it does not lower the surface tension of benzene and similar liquids against air. When a large drop of water is poured into a benzene solution of dammar a visible film, wrinkling in folds as the drop is distorted, is formed in a few seconds. Freezing point determinations show a molecular weight not far from that indicated by the formula. Probably dammar is sufficiently polar to function as an emulsifying agent.

The proteins and soaps are polar and in addition their molecules form large colloidal aggregates hence the greater strength of their films in emulsions. There is really a great difference in the emulsifying powers of the soaps and of the lower alcohols but the important point is that certain substances in true solution do form distinct emulsion films.

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IODINE AS AN EMULSIFYING AGENT

By Harry N. Holmes and H. A. Williams

To illustrate the Nernst distribution law some ether was shaken with water to which had been added iodine and potassium iodide. (W. H. Chapin in this laboratory.) An emulsion formed and slowly creamed. The cream, which was very viscous, lasted for several days. Since the cream rose it represented an emulsion of ether in water, or, to be exact, of wet ether in ethereal water.

No emulsion could be prepared from ether and water alone so it was evident that the emulsifying agent was iodine, potassium iodide or potassium tri-iodide. We failed to secure an emulsion on shaking ether with a freshly prepared aqueous solution of potassium iodide yet an old solution enabled us to make a very poor emulsion. Probably a very little iodine was released by the action of light or by the action of the carbon dioxide and oxygen of the air. This led us to try iodine alone as the emulsifying agent for the system ether-in-water. Good emulsions were obtained, improving as the concentration of iodine in the wet ether was increased up to one per cent.

In attempting to prepare emulsions of 75 per cent by volume we found very little value in a 0.05 per cent solution of iodine in wet ether. A very unstable 50 per cent emulsion was, however, prepared with this concentration of iodine. At a 0.1 per cent concentration of iodine a fair 75 per cent emulsion was made while a 1.0 per cent iodine solution showed the maximum emulsifying power in this system.

That potassium tri-iodide could not be held responsible for emulsification was shown by trying to emulsify ether in an aqueous solution of iodine and potassium iodide containing an excess of the iodide. This gave us a much poorer emulsion than a similar solution containing a good deal less potassium iodide. Potassium chloride did not break such an emulsion.

$$KI + I_2 \rightleftarrows KI_3$$

Increasing the amount of potassium iodide present drives the above equilibrium to the right, making *more* tri-iodide and *less* free iodine. Consequently iodine alone must be the emulsifying agent. Neither bromine nor chlorine showed any such emulsifying properties nor did such salts as sodium iodide, calcium iodide, sodium bromide, potassium chloride and sodium sulfate.

J. von Amann[1] observed that red-brown solutions of iodine in carbon disulfide, carbon tetrachloride, chloroform and in petroleum showed practically no ultra-microscopic particles. He found some of these colloidal aggregates in amyl acetate solutions of iodine and many in an amyl alcohol solution. It is noteworthy that we failed to secure good emulsions of carbon disulfide, carbon tetrachloride, and chloroform in water using iodine as the emulsifying agent while good emulsions were made by dispersing amyl alcohol, amyl acetate, or ethyl acetate in water with the aid of iodine.

Evidently a film of colloidal aggregates of iodine formed at the liquid—liquid interface. This must be a Gibbs adsorption film rather than a mere precipitation film for we were able to saturate ether with water and water with ether and proceed to make good emulsions after iodine had been added to either or both of the solutions.

Hildebrand holds that the brown color of certain iodine solutions is due to formation of compounds.

To prove that iodine did concentrate at the liquid—liquid interface—we saturated 20 cc. of water with iodine and added, in small amounts, 10 cc. of ether, shaking vigorously after each addition. In this way a good emulsion was built up, creaming over night. The water layer underneath no longer gave the starch test for free iodine. Obviously the iodine had all gone into the cream where we had the greatest area of interface. The Nernst distribution law held, as expected, when we cautiously poured a layer of 10 cc. of ether on 20 cc. of water saturated with iodine (no shaking and no emulsifying) and allowed to stand several hours. Iodine was found distributed in both layers.

All the emulsions were of the oil-in-water type. When the dispersed liquid was the lighter the cream rose, and when the dispersed liquid was the heavier the cream sank. Drop dilution tests confirmed this conclusion. It is difficult to account for this type of emulsion considering that the emulsifying agent, iodine, is more soluble in the dispersed phase than in the continuous phase. By the usual rule the opposite type, water-in-oil, should be obtained. Nor can we readily suggest polarity of the emulsifying agent when this agent is an element.

It may be that the aggregates of iodine in the film receive some sort of wedge shape due to penetration of ether between aggregates on one side of the film and of water between aggregates on the other side.

That the drops were negatively charged was shown by the greatly superior precipitating power of positive polyvalent aluminum ion as compared with univalent positive ions or with any negative ion.

As might be expected, it is easier to make an emulsion (using iodine as the emulsifier) with a pair of liquids showing considerable but not complete miscibility than with too much less miscible liquids. This is

[1] *Kolloid Z.*, 6, 235 (1910); 7, 67 (1910).

merely another way of stating that the lower the interfacial tension between the two liquids the easier emulsification is secured. In the case of iodine this influence is modified by the von Amann phenomenon previously mentioned.

The use of iodine as agent enables us to prepare 75 to 80 per cent (by volume) emulsions of ether, ethyl acetate, amyl acetate, or amyl alcohol in water. These creamed to richer emulsions and broke after a few days. Hand shaking was used and, in fact, seemed preferable to other types of agitation.

As a good example of one of these emulsions we suggest the preparation of an ether-in-water emulsion. To 10 cc. of water (saturated with ether) add slowly with vigorous hand shaking 30 cc. of a one per cent solution of iodine in ether (saturated with water). When this is carefully prepared the emulsion is almost jelly-like.

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THE ORIENTATION OF MOLECULES IN THE SURFACES OF LIQUIDS

By William D. Harkins

The importance of a comprehensive theory of surfaces and of surface energy lies in the fact that surfaces are abundant in material systems, whatever their nature. Even fluid systems, which are at first sight entirely homogeneous are found upon closer examination to hold great numbers of minute particles, each of which has a surface or interface. Interfaces are of particular significance in living organisms, since the motion of an organism as a whole is evidently brought about by transformations of one kind or another of the interfacial energy resident in it. The term surface unfortunately implies the entire absence of a third dimension in space, that of thickness, but physical surfaces and interfaces, sometimes designated as phase-boundaries, although they are exceedingly thin, commonly have a thickness as great as the sum of the diameters of several atoms, or a distance of the order of a millionth of a millimeter (1 $\mu\mu$), which is by no means negligible. At many interfaces films or membranes collect, and these are of particular importance in biological systems, particularly in the human body itself.

The ordinary observation of large scale objects, such as logs or ships, as they lie upon the surface of a body of water, indicates that these objects exhibit a characteristic orientation with respect to the surface. Thus logs, when not too closely crowded together lie flat upon the water, that is the longitudinal axis is parallel to the surface. However, if one end of each log is loaded with a mass of iron or brass of the proper weight, it floats upon the surface and the longitudinal axis becomes vertical. If there is just a sufficient number of logs, the surface becomes covered with a single layer of vertical logs with their sides more or less in contact, while with any greater number, bunches of logs are found raised above the common level in certain places. If the number is smaller, a part of the surface remains uncovered. (These phenomena were illustrated by the use of a large number of cylindrical sticks of wood 3 mm. in diameter and 14 cm. long, weighted by a small cylinder of brass placed at one end. These were thrown upon the surface of the water in a large glass cylinder. This is represented in a diagrammatic way in Fig. 1. One of the vertical sticks was taken from the water, the brass weight removed, the stick dropped upon

a vacant space upon a water surface. At once this assumed a horizontal position, thus exhibiting another type of orientation.)

It is well known that the molecules or ions which make up a crystalline solid are arranged in an orderly way. A certain type of orderly

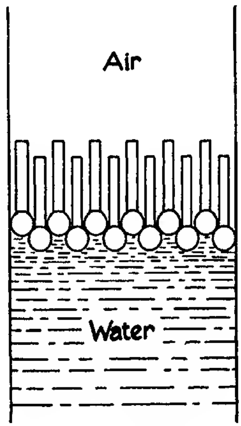

FIG. 1.—Weighted logs floating on water.

array of very long and highly symmetrical molecules is found also in certain liquids, which are said to contain liquid crystals. Ordinary liquids are supposed to be characterized by a complete disorder in the arrangement of their molecules, but it is not improbable that this dis-

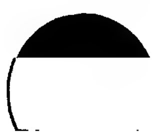

FIG. 2.—Oriented sphere. Indicates that dissymmetry of forces may cause the orientation of an object which possesses spatial symmetry.

order has been overemphasized. For example in organic liquids of the type of acetic acid, the molecule of which consists of the polar (heteropolar) carboxyl group, and the "non-polar (homopolar) methyl group, there is some evidence of molecular association, presumably a type of orientation in which two or more polar groups come close together (see upper phase in Fig. 3). The theory that the molecules in the surface of a liquid are oriented in a characteristic fashion, is of

comparatively recent origin. It seems peculiar that the birth of so obvious a conception should have been so long delayed, but it is probable that this is due to the general habit of considering molecules as spherical, even when their formulæ are highly elongated. In such cases the former conception was that it would roll itself up into a sphere. It is obvious that even in a dissymmetrical field of force, such as may be assumed

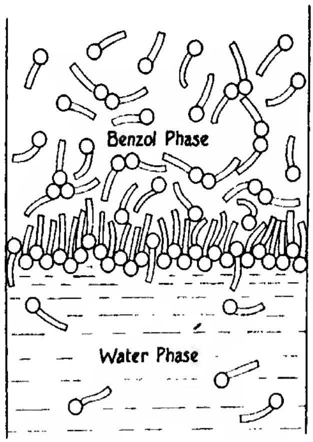

FIG. 3.—Butyric acid distributed between water, benzol and the interface between the two.

to exist at the surface of a liquid, a molecule which is a perfectly symmetrical sphere could exhibit no orientation. However, even in a uniform gravitational field a perfect sphere may orient itself provided its mass is not uniformly distributed, as will be seen if a sphere is weighted on one side, so that even if all molecules were spherical molecular orientation would not be at all impossible (Fig. 2).

The conception and development of the orientation theory of surface structure is due to the work of three investigators.[1] The first of these, Hardy, expressed his early contribution in the two following paragraphs:

"The corpuscular theory of matter traces all material forces to the attraction or repulsion of foci of strain of two opposite types. All systems of these foci which have been considered would possess an unsymmetrical stray field—equipotential surfaces would not be disposed about the system in concentric shells. If the stray field of a molecule that is of a complex of these atomic systems, be unsymmetrical, the surface layer of fluids and solids, which are close-packed states of

[1] The present paper describes the work of the writer. The important work of Langmuir is described in the following references: *J. Am. Chem. Soc.*, 39, 1848 (1917); *Chem. Met. Eng.*, 15, 468 (1916).

matter, must differ from the interior mass in the orientation of the axes of the fields with respect to the normal to the surface, and so form a skin on the surface of a pure substance having all the molecules oriented in the same way instead of purely in random ways. The result would be the polarization of the surface, and the surface of two different fluids would attract or repel one another according to the sign of their surfaces. (Hardy, 1912.)

"If the field of force about a molecule be not symmetrical, that is to say, if the equipotential surfaces do not form spheres about the centre of mass, the arrangement of the molecules of a pure fluid must be different at the surface from the purely random distribution which obtains on the average in the interior. The inwardly directed attractive force along the normal to the surface will orientate the molecules there. The surface film must therefore have a characteristic molecular architecture, and the condition of minimal potential involves two terms—one relating to the variation in density, the other to the orientation of the fields of force." (Hardy, 1913.)

In connection with the orientation theory the principal effort of the writer and his associates has been to obtain extensive and fundamental evidence in its favor. The evidence secured by twelve years of work is largely that presented by the energy relations which are exhibited at interfaces. While fundamentally of a very simple character, the number of numerical magnitudes involved is so large as to be confusing to one who does not have them well in mind. The purpose of the present paper is, therefore, to bring out in a simple way, and largely by the use of models and diagrams, the most striking points in the proof, without a repetition of the great mass of data which may be found in the original papers. To further increase the simplicity of the discussion it seems advisable to first present the principal items in the story of the development of the theory in this laboratory.

The work arose in what may seem to be an accidental way. In 1907 von Lerch, at that time working in Nernst's laboratory, observed that the capillary height method as used by him, indicated a sudden decrease in the interfacial tension between water and benzol at the neutral point if the aqueous phase contains acid (HCl) and if to this a base (NaOH) is added. In 1909 Haber and Klemenciewicsz found that the electromotive force between the two phases also exhibited a great change in the vicinity of the neutral point. Now it was known that an active muscle gives an acid reaction, due to the production in it of lactic and other acids, while a muscle at rest is slightly alkaline. Also between a muscle in motion and one at rest there is a certain, though minute electromotive force. A comparison of these facts with those concerning the system water-benzol suggested to Haber that since in the latter case the surface tension seemed to vary rapidly at the neutral point, this should be true in the muscles also, and this change in sur-

face tension might well be responsible for the motion of the muscles. Professor Haber suggested to the writer as a first step in the support of this idea that von Lerch's work be repeated. Unfortunately for the simple analogy this repetition showed that the water-benzol interfacial tension is not largely decreased by the addition of a base, though such a decrease was found to be effected in many cases when more complex organic substances are used. If acid is put in the aqueous phase it is to be expected that H^+ ions, on account of their high velocity would pass into or beyond the interface more rapidly than the chlorine ions, while with the base, this would be true of the negative OH^- ions. Thus, at least for a time, it may be supposed that there is a certain orientation of the H^+Cl^- or Na^+OH^-.

In thinking over this problem in 1912 the writer came to the conclusion that many of the phenomena at interfaces could be more easily explained on the basis of the actions of molecules. Thus the idea arose, on the basis of the principle "like dissolves like" that if a substance of the type of butyric acid were present in the system water-benzol, the solubility in the water would be due to the carboxyl group ($-COOH$), and that in the benzol to the hydrocarbon chain ($CH_3CH_2CH_2CH_2-$). It was well known that an increase in the length of the hydrocarbon chain decreases the solubility of the acid, and increases the fraction which enters the organic phase. It seemed, therefore, that in any such case the concentration of the acid should be highest at the interface when equilibrium is reached, since there the carboxyl group could dissolve in the water, while the hydrocarbon group could dissolve in the organic phase.

A later test of this specific idea proved that when one molecule of butyric acid to 270 of water is present in the aqueous phase, one molecule of acid to 34 is present in the organic phase while at the interface the concentration of butyric acid in the monomolecular film is almost as high in two dimensions as in pure butyric acid, since 2.79×10^{14} molecules are present per sq. cm. of area (Fig. 3). Thus the solubility in the interface reaches very nearly the highest possible value.

The idea of molecular orientation in surfaces seemed to the writer an entirely novel one, but the possibility existed that a similar statement might exist in the literature. An extensive search proved this to be the case, since near the end of a long paper which did not speak otherwise of orientation Hardy's statements, as cited above, were found.

The discovery of the first of these paragraphs indicated that the writer's idea was not so novel as it had seemed, but on the other hand, since Hardy had presented no evidence in favor of his idea, it emphasized the need for the collection of extensive experimental data and for its interpretation in terms of the new idea. The need for such evidence has been brought out by Edser, practically the only objector to the theory during the twelve years of its existence, who calls attention

to the pertinent objection that the motion of the molecules is so rapid as to make orientation improbable. This objection has been considered by the writer also, but with a different conclusion, as is indicated by the following quotation:[2]

The most fundamental characteristic of a surface is the unlikeness of its two sides and the resultant dissymmetry of the molecular forces involved. If the molecules in the surface are not entirely symmetrical this lack of balance in the forces must result in their orientation to a smaller or a greater degree. Since, however, the heat motion of the molecules is very great at ordinary temperatures, it might well be that the orientation would be thus so greatly reduced as to produce no noticeable effect upon ordinary surface phenomena. Thus, not only are the molecules in the surface of water vibrating with extreme rapidity, but their orientation is also disturbed by the escape of about 7×10^{21} molecules from each square centimeter of surface every second (at 20° C.). Not only is this the case, but if the water and its vapor are in equilibrium, molecules to the same number jump back into the surface in the same minute interval of time.* It is thus seen that the idea that there are forces which would produce orientation in a static system is not sufficient to demonstrate that such orientation has an appreciable magnitude. For such a demonstration, definite experimental evidence is necessary.

The first work begun in this connection was on the adsorption at interfaces between liquid phases, but the principal interest of the laboratory became focused upon a comparison of the surface energy values at surfaces and interfaces. A considerable number of such data had been determined by Hardy, but he had not indicated any relation to the idea of orientation. Moreover, practically all of the interfacial tensions found in the literature were discovered to be extremely inaccurate, and such values give an extremely uncertain basis for a new theory. On this account somewhat extensive investigations were carried out in order to increase the precision of both the theory and the practice of the drop weight and the capillary height methods.

While the primary attention was thus given to interfaces between

[2] "Colloidal Behavior," Vol. I, p. 149 (1924).

* Since there are about 10^{15} molecules of water in 1 sq. cm. of surface, this means, if we consider the area covered by this molecule, that 7,000,000 times during 1 second the molecule in this area at the instant would jump out into the vapor, and (also on the average) a molecule would fall from the vapor, upon this area 7,000,000 times. (If air is present at atmospheric or a higher pressure the number of molecules escaping is undoubtedly reduced somewhat by the bombardment of molecules of air.) Since there would also be an enormous number of exchanges between the surface of the liquid and the molecular layer just below, it will be seen that the surface is in anything but a static condition. Thus, if there is to be any appreciable degree of orientation of the molecules on the average, the time of orientation should fall considerably below $\frac{1}{20,000,000}$ second. Since the data on surface energy indicate a marked degree of orientation in most liquids at this fraction of their critical temperature (0.487), it would seem that the time of orientation is of the order of $\frac{1}{100,000,000}$ second or less, which seems entirely plausible when the rapidity of rotation of such a system is considered.

liquids, it was natural that films of oil on water should be considered. Thus in speaking of the spreading of oleic acid on water the writer expressed the germ of the theory: "COOH of acid down because both acid and H_2O associated and polar." (Quotation from the lecture notes of George L. Clark as taken in March, 1914.) This is of interest since it is the earliest record thus far found to give the direction in which the molecules are oriented at any surface.

It is well known that work must be done in order to form a surface on a liquid, as is indicated by the arrangement (Fig. 4) suggested by Maxwell. This represents a liquid film with two surfaces. If the

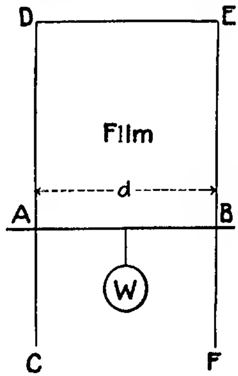

FIG. 4.—Film stretched on a frame.

distance AB is one-half cm., and this wire is pulled one cm. downward, then, since there are two surfaces, one sq. cm. of new surface will form. Thus, if the pull of each surface is γ dynes per cm. (γ = surface tension), then the work done to produce a unit area of new surface is γ ergs, which is the value of the free surface energy. This work aids the molecules to rise into the surface. The energy thus supplied in the form of work is never sufficient to form a new surface, so the additional energy is supplied from the kinetic energy of motion of the molecules themselves. This energy is designated as the latent heat (l) of the surface. At 0° C. the average translational energy of a molecule of any gas is 5.62×10^{-14} ergs, or at any temperature it is $2.06 \times 10^{-16}T$ ergs. Now it is of interest that in order to rise into a surface, thereby forming a new surface area, the average molecule of an unassociated liquid gives up 2.96×10^{-16} ergs of kinetic energy, which is thus transformed into energy of the potential type, that is energy of position in the surface. Thus, in order to get into the surface a molecule needs to pay in terms of energy 45% more than the average wealth of a molecule in translational energy. This indicates

that on the whole only the more rapidly moving molecules are able to rise into the surface.

Whether or not the point of view is that indicated in the last paragraph, it is evident that the principal procedure which needs to be understood in connection with a theory of the surface of a liquid, is that of the lifting of molecules in order to form a new surface area. So long as the area remains unchanged at constant temperature, a molecule from below is not able to enter the surface unless it displaces another molecule from this position. Since the molecule which enters the surface gains on the average only the energy which is given up by the one displaced, there is no change which affects the ordinary measurements of surface energy, which relate only to the appearance or disappearance of a new surface area. If we again make use of the illustration in which a log, weighted by metal at one end, has an average density somewhat less than water, the following procedure may be

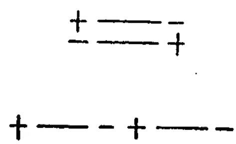

FIG. 5. -Simple sets of electrical bipoles.

imagined. The log is at first entirely underneath the surface of the water. When it rises into the surface (Fig. 1) the lightest end, that is the end which requires the least work to lift it, is raised above the surface, while the weighted or heavy end remains buried in the liquid. If the log is sufficiently near the surface in the beginning the heavy end does not need to be lifted, and may even fall.

It may be supposed that many molecules of the unsymmetrical type, possess a weighted and a light end. A good example of this is that of an organic molecule such as butyric acid, already cited, with the light or non-polar, and the heavy, or polar end. Here the terms light and heavy do not refer to the weight due to gravitation but to lightness and heaviness with respect to the electromagnetic forces which give rise to molecular attraction.

There is much evidence which indicates that a complete atom is electrically neutral, and that it consists of a minute nucleus, with a diameter of the order of one-ten-thousandth of that of the atom. This nucleus possesses a net positive charge of electricity, which, in terms of the charge on a single negative electron as unity, is numerically equal to the atomic number. Thus the nucleus of a sodium atom, atomic number 11, has a net positive charge of 11. To give the complete neutral atom, 11 negative electrons may be supposed to rotate in orbits around

the positive nucleus. In general, if in each of two bodies the positive and the negative electricity are located in different positions, there is an electrical attraction between the two, though there may be positions in which the resultant effect is a repulsion. Also, if equal charges of opposite sign are uniformly distributed upon the surfaces of two concentric spheres, there is no electrostatic effect outside the outer sphere. This latter case does not arise in the case of atoms, whose electronic constituents are extremely small. However, even here, the symmetry of distribution of the negative electrons around the central positive

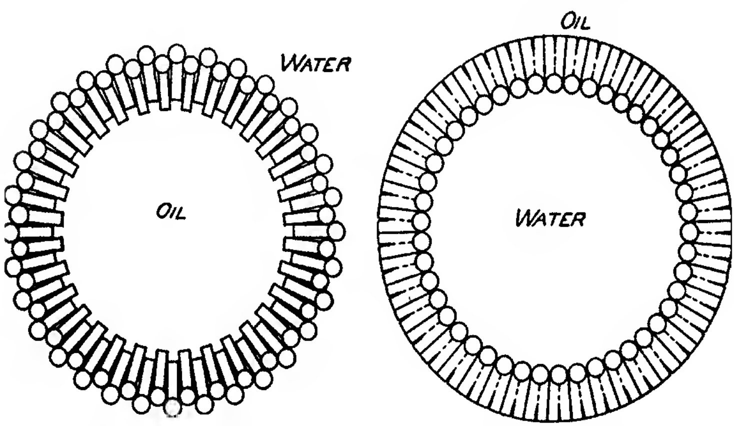

FIG. 6.—Molecules of the polar-nonpolar type with polar ends weighted toward water. (a) Water outside. (b) Water inside, oil outside. This occurs when there are two hydrocarbon chains and COO⁻ groups attached to a single atom of a bivalent metal. If, however, there is only one hydrocarbon chain this may be taken to represent a drop of water surrounded by an octyl alcohol film floating in air, as described in the text. Both diagrams are highly conventionalized as the molecules in the film are undoubtedly moving with great rapidity.

charge has a marked effect upon the field of force outside the atom. For example the most symmetrical distributions of electrons are found in the atoms of the zero group: helium, neon, argon, krypton, and xenon. The attraction between such symmetrical or non-polar atoms is so slight that the gases cannot be liquefied at ordinary temperatures. A molecule of gaseous sodium chloride is, on the other hand, highly polar, since its sodium ion is positively, and its chlorine ion, negatively charged. In polar molecules or groups of atoms, the ends of the electrical bipole are relatively far apart. Such electrical bipoles possess a specifically high attraction for each other. (Fig. 5.) The polarity of a saturated hydrocarbon chain is small. The most common polar groups in organic

compounds are $-OH$, $-COOH$, $-CHO$, $-CN$, $-CONH_2$, $-SH$, $-NH_2$, $-NHCH_3$, $-NCS$, $-COR$, $-COOM$, $-COOR$, $-NO_2$, $-\overset{\displaystyle H}{\overset{|}{C}}=CH_2$, $-C\equiv CH$, or groups which contain oxygen, nitrogen, sulfur, iodine, bromine, chlorine, double or triple bonds. Water is a highly polar liquid, though much less polar than molten sodium chloride, so the above groups are highly

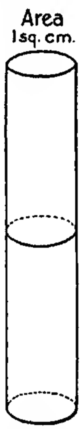

FIG. 7.—Bar of unit cross section.

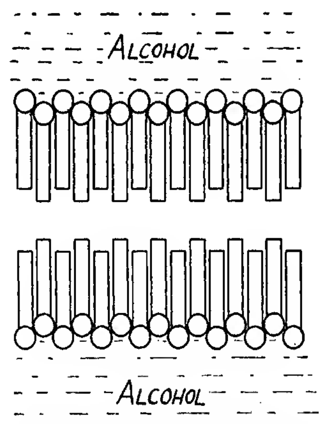

FIG. 8.—Orientation of molecules when a column of alcohol is broken apart to give two surfaces.

attracted by water. Thus the polar end of the molecule of an alcohol or an organic acid is highly weighted toward water. This is illustrated by Fig. 6b, in which a minute spherical drop of water is floating in air. Surrounding this is a monomolecular film of octyl alcohol, with the polar or electromagnetically weighted end toward the water and the non-polar end lifted away from the water. Heie it must be remembered that up or down in the sense of the relative position of the earth, has no appreciable effect. The octyl alcohol molecules have an orientation similar to that of the trees on the earth, but with a more general distribution.

Suppose that we have a glass cylinder filled with octyl alcohol and let the liquid have unit cross sections (1 sq. cm., Fig. 7). Let us assume that we can break the liquid column at a designated level (what corresponds to this may be done in a different way). In general a bar will break at its weakest point, so what occurs is that first the molecules of octyl alcohol orient themselves as the break begins, and finally only

the non-polar ends of the molecules, whose attractions for each other are weak, have to be pulled apart. (Fig. 8.) To the molecules the downward direction is toward the body of the alcohol, so at each of the two surfaces thus formed the polar groups turn toward the liquid. In this way 2 sq. cm. of surface is formed, in which the very outer surface consists of non-polar groups. That is, in forming the surface, only the light end of each molecule had to be lifted. If, on the other

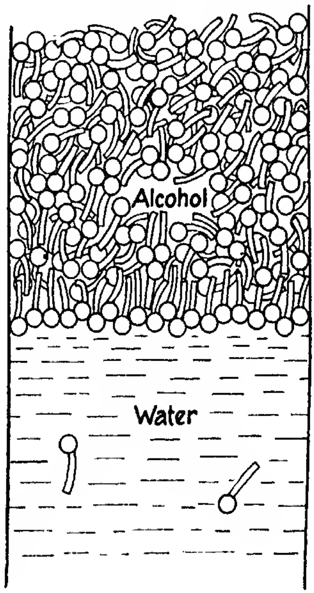

FIG. 9.—Octyl alcohol over water showing orientation of alcohol molecules in the interface.

hand, octyl alcohol is pulled away from water, polar hydroxyl groups must be pulled away from polar water (Fig. 9) provided the molecules are oriented as the theory indicates. In entire accordance with the theory it is found that the work required to pull octyl alcohol away from water is 91.8 ergs per sq. cm. of interface, which is more than 80% higher than the work done in pulling the alcohol itself apart. That is the work of adhesion (W_a) is more than 80% higher than the work of cohesion (W_c).

Even more striking than this is the fact that in the case of alcohols the work required to pull the alcohol away from water is nearly independent of the length of the hydrocarbon chain, which would be ex-

tremely difficult to explain if orientation is not assumed. For example the increase of the hydrocarbon chain from 1 carbon atom in methyl alcohol to 8 in octyl alcohol, reduces the work of adhesion only from 95.5 to 91.8, or by less than 4 per cent. In methyl alcohol the polar hydroxyl group occupies about one-half of the total volume of the alcohol, while in octyl alcohol it occupies only about one-ninth, so in the latter the polar groups would have much less chance of approaching the water surface if orientation were absent. A former paper (Colloidal Behavior, pg. 159) summarizes the further relations as follows:

"A consideration of the relations at the interface between octyl alcohol and water from the standpoint of the dimensions found for alcohol molecules in films on water, is of interest. It will be seen later that the number of alcohol molecules per sq. cm. is about 3×10^{14}, while it is easily calculated that for symmetrical water molecules the number is about 10×10^{14}. A simple calculation shows that the work necessary to pull the alcohol from water at the interface between the two, is about 30×10^{-14} ergs, while in separating water from water it is about 15×10^{-14} ergs per molecule of water on one side in a plane. These energy values seem to indicate the probability that at the interface the —OH groups of the alcohol are close to several molecules of water. Also they suggest that the fact that the work of separation of the alcohol from water (91.8) is smaller than that of water from water (145.8), is not due to the relative smallness of the attractions around the hydroxyl group of the alcohol, but to the relatively small number of such groups as compared with the number in the surface of water, the ratio being only about 1 to 3.3.

"The molecule of octane contains 26 atoms. The introduction of a single oxygen atom into this molecule increases the work of surface cohesion in water by only 26%, but it more than doubles the work of adhesion, actually increasing the value by 111%, which is a remarkably high effect for the addition of a single atom per molecule. The values for caprylic acid, with 8 carbon atoms, are almost identical with those for octyl alcohol, since the work of cohesion for caprylic acid is 57.6 ergs, while its work of adhesion toward water is 93.7 ergs per sq. cm. Thus it will be seen that in the case of *non-symmetrical* molecules, such as those of octyl alcohol ($C_8H_{17}COOH$), caprylic acid ($C_7H_{15}COOH$), and mercaptan (C_2H_5SH), *the adhesional work* (W_a) *is determined by the strongest electromagnetic fields in the molecule, while the tensile free energy* (W_c) *is determined by the weakest fields*, so for unsymmetrical molecules the work of adhesion is relatively high, and the work of cohesion low. In the case of entirely symmetrical molecules there could be no orientation, though a molecule which is symmetrical in the gaseous state may be expected to become less symmetrical when placed in the non-uniform field at the surface of a liquid. An increase in symmetry, without a change in the compo-

sition of the molecule, is found to increase the work of cohesion, and to decrease the work of adhesion toward water, which is exactly in accord with the hypothesis that the molecules in the surfaces are oriented, since an increase of symmetry not only reduces the extent of the orientation, but it also decreases the effect of such an orientation upon the energy values. Thus it is only when an organic molecule is

TABLE I

EVIDENCE FOR MOLECULAR ORIENTATION IN SURFACES

Illustrates the Small Effect on the Cohesional, and the Large Increase of the Adhesional Work which accompanies a Decrease in the Symmetry of Molecules of the Substance. (In the table the substance in the upper phase is listed above, and that in the lower phase, below the line. Energy values in ergs per sq. cm. of cross section of the bar of liquid disrupted.)

Octane	Octyl Alcohol	Octylene	Heptylic Acid	Heptane
43.5	55.1	44.7	56.6	40.2
Octane	Octyl Alcohol	Octylene	Heptylic Acid	Heptane
Octane	Octyl Alcohol	Octylene	Heptylic Acid	Heptane
43.8	91.8	72.9	94.8	41.9
Water	Water	Water	Water	Water

CCl_4	CCl_4	$(CH_3)_3CCl$	$(CH_3)_3CCl$
53.5	56.2	39.2	68.6
CCl_4	H_2O	$(CH_3)_3CCl$	H_2O
$CHCl_3$	$CHCl_3$	$(CH_3)_2CH_2CH_2Cl$	$(CH_3)_2CH_2CH_2Cl$
54.3	67.3	43.8	70.3
$CHCl_3$	H_2O	$(CH_3)_2CH_2CH_2Cl$	H_2O
CH_2Cl_2	CH_2Cl_2	$(CH_3)CH_2CH_2CH_2Cl$	$(CH_3)_2CH_2CH_2CH_2Cl$
53.0	71.0	47.0	80.8
CH_2Cl_2	H_2O	$(CH_3)CH_2CH_2CH_2Cl$	H_2O

CS_2	C_2H_5SH
62.8	43.6
CS_2	C_2H_5SH
CS_2	C_2H_5SH
55.8	68.5
H_2O	H_2O

Note that Octane and heptane are symmetrical with reference to the forces around the molecule, and that CCl_4, CS_2, have highly symmetrical molecules.

Octyl alcohol, and heptylic acid have highly unsymmetrical molecules, and their polar groups are highly polar (hydroxyl or carboxyl).

Mercaptan has an unsymmetrical molecule, but its —SH group is less polar than carboxyl. In the chlorine compounds chlorine is more polar than the hydrocarbon chain.

The data indicate that the molecules are oriented at the surfaces, and that at the interface with water the polar groups orient toward the water.

moderately symmetrical with respect to the electromagnetic field (largely electrical) which it produces, that the work of cohesion can become greater than that of adhesion.

It is thus found that the value of W_a-W_c, which will be designated as S, is highly dependent upon lack of molecular symmetry for its high positive values, and upon the presence of such symmetry for its high negative values. For the highly unsymmetrical alcohols S is about 50, while for the symmetrical acetylene tetrabromide it is —5.7, and for methylene iodide (CH_2I_2) it is —26.5. It will be shown later that S is an important function in connection with spreading, so it may be called the "spreading coefficient." In general liquids will spread when S is positive, and will not spread when S is negative.

Table 1 gives the adhesional work for a number of different liquids. Let us consider carbon disulfide and ethyl mercaptan. The cohesional work in the former is much higher, 62.8 instead of 43.6, yet the attraction between water and carbon disulfide (adhesional work = 55.8) is much less than that between water and mercaptan (68.5).

The former has a symmetrical molecule, and the latter an unsymmetrical. The hydrosulfide group is evidently more polar than the divalent sulfur atom, but when the mercaptan lies in contact with the water, most of the hydrosulfide groups are turned toward the water, and when they are pulled from it, the polarity of the group is evident, in the high value of the adhesional work. The = S group, not being so polar, gives a considerably smaller value, which is 12.7 ergs less. However, the attraction between the sulfur of carbon disulfide and water, and also that between the sulfur in the different molecules of the carbon disulfide itself, is much greater than the attraction between hydrocarbon groups such as C_2H_5—. Now when a bar of carbon disulfide is pulled apart to make two surfaces, sulfur must be pulled away from sulfur, so the cohesional work, and also the total cohesional energy are relatively high, the former having a value of 62.76 ergs per square centimeter. However, when the mercaptan is pulled apart, sulfur (—SH) does not need to be pulled from sulfur, but the sulfur turns under the surface, and only the hydrocarbon groups have to be pulled apart, so the work of separation is low (only 43.6).

A comparison of the halogen derivatives is also instructive. The cohesional work for carbon tetrachloride, chloroform, and methylene chloride is almost the same (53.32, 54.26, 53.04), but the adhesional work toward water rapidly increases in the order given (56.16, 67.30, 71.0). Here the increasing polarity is counterbalanced by the concomitant increase of dissymmetry, which allows the less polar parts of the molecules to be oriented into the surface. At the interface, however, it is the most polar part which is turned into the interface, so the effects add together instead of subtracting. Also, the adhesional work

for isobutyl and tertiary butyl chloride are practically as high as in the case of methelene chloride, since the chlorine is turned toward the water, but the cohesional surface work drops to very low values, 43.88 and 39.18 ergs per square centimeter.

Both carbon tetrachloride and ethylene dibromide give the same value for the cohesional work as for the adhesional work, but, as the number of bromine atoms in the compound increases (acetylene tetrabromide), the cohesional work becomes the higher. These compounds have very symmetrical molecules.

A comparison of isopentene with trimethyl ethylene and of octane with octylene shows that the introduction of a double bond increases the cohesional work very slightly and the adhesional work very greatly, especially in the latter case, where the double bond is at the end of the molecule. These facts are again exactly in accord with the orientation theory. For octane the cohesional work is 43.54, while for octylene it is almost the same, or 44.66, so the introduction of the double bond has little effect. The value of the adhesional work for octane is practically the same as that for the cohesional work, but the addition of the double bond in octylene raises the value by about 60 per cent.

In a later paper (Harkins and Cheng) it is shown that the total adhesional energy and the total cohesional energy exhibit, in general, exactly the same relations as those given above for the work involved, except that in the former case, all of the energy values are greater. Thus, the addition of 1 oxygen atom to the 26 atoms already present in octane to form octyl alcohol increases the cohesional energy by only 2%, but the adhesional energy by 65%. The cohesional energy of ethylene dibromide is, on account of the symmetry of the molecule, much greater than that of isomeric but unsymmetrical ethylidene dibromide.

The general effect of double bonds near the end of the molecule is to increase the adhesional, but not the cohesional, energy. The so-called double bonds in benzol are distributed with such symmetry that they increase both the cohesional and the adhesional work and energy.

Molecular Orientation as Exhibited by the Relations Between the Energy of Surface Formation and That of Vaporization

While inside a liquid a molecule moves as readily in one direction as in another, since the forces of molecular attraction, which give rise to the cohesion of the liquid, act alike in all directions.[a] However, if the molecule approaches sufficiently close to a phase boundary this is no longer the case. Thus if the boundary is that between liquid and vapor

[a] Provided the time interval considered is not too minute.

the attraction toward the vapor phase is here less than that toward the liquid phase. This makes it necessary that energy be used if a molecule is to be lifted into the surface. The energy (e_s) supplied in order to create a new surface equal to the area of a single molecule comes from the kinetic energy of molecular motion and from the work done in extending the surface. The energy contributed by the molecule is designated as the latent heat (l_s) of the surface, while the work utilized is equal in magnitude to the free surface energy (γ). Thus

$$e_s = l_s + \gamma$$

or for 1 sq. cm. of surface

$$E_s = L_s + \Gamma$$

In the case of normal organic liquids, liquid oxygen, liquid nitrogen, and other similar liquids the following interesting relation is found to hold: Whenever a molecule moves from the interior of the liquid into its surface in such a way as to form a new surface the average amount of its kinetic energy which is converted into potential energy is equal to 144% of the mean translational kinetic energy of a gas molecule at the same temperature. This indicates that in general the energy required to be supplied by the molecules is greater than the kinetic energy of the average molecule. The free energy of the surface is the difference between the total energy, which depends upon the structure of the surface, and the latent heat of the surface, which is conditioned by the relation expressed above. It is easy to see why, on this basis the free surface energy decreases rapidly as the temperature increases. Unless the critical temperature is approached too closely the total surface energy (e_s) usually either remains constant, or more commonly decreases slightly. The contribution (l_s) from the kinetic energy of molecular motion increases as the temperature, so the difference $\gamma = e_s - l_s$ must decrease rapidly as the temperature rises.

The principal difficulty for an analogy between the action of gravitational forces upon the earth, and surface forces, lies in the extreme thinness of the layer, known as the surface, inside which the forces differ in magnitude. This is of the order of 1 μμ (or 10^{-7} cm.) in thickness. Suppose that a number of logs of a wood heavier than water lie on the bottom of a pond. Let a heavy iron weight be now attached to one end of each of the logs. If the pond is more shallow than the depth of the pond it is evident that the lighter or wooden end of each of these objects may be lifted into the surface while the iron end is still left resting upon the bottom. However, if each such object is taken entirely out of the water, the heavy end as well as the light, must be lifted. Corresponding to this, when the surface of an alcohol or other substance which consists of polar-nonpolar molecules, is formed, only the electromagnetically light or

nonpolar end has to be lifted into the outer part of the surface, while if the liquid evaporates the electromagnetically heavy or polar end must also be lifted. Thus for molecules of this type the energy of surface formation should be much smaller than the energy or "heat" of vaporization (λ). With an entirely symmetrical model there is no heavier and no lighter end, so with symmetrical molecules the energy of surface formation should be a much larger fraction of the heat of vaporization. This fraction ($\frac{e}{\lambda}$) exhibits values which constitute extremely strong evidence in favor of the orientation of unsymmetrical molecules in surfaces and in interfaces in general. As a molecule of the unsymmetrical type that of ethyl alcohol may be chosen, while that of carbon tetrachloride may be taken to represent symmetry. At a corresponding temperature of 0.7 only 0.19 as much energy is needed to carry a molecule of alcohol into the surface as to carry it completely into the vapor, while for carbon tetrachloride the corresponding value is very much higher (0.45). At 0.8 as the temperature, the values are 0.25 and 0.53; and at 0.9, they are 0.4 and 0.7; so in each case the value is very much lower for the unsymmetrical molecule, which is exactly what the theory predicts.

TABLE 2

Liquids Arranged in the Order of the Ratio of the Energy Necessary to Carry a Molecule into the Surface to That Required for Complete Vaporization, and Presumably in Order of Increasing Symmetry in the Surface

(Corresponding Temperature = 0.7)

Molecule	e/λ	e/j
Class 1		
1. Methyl alcohol	0.164	0.191
2. Ethyl alcohol	0.186	0.228
Class 2		
3. Water	0.282	0.372
4. Acetic acid	0.336	0.474
Class 3		
5. Ethyl acetate	0.397	0.606
6. Methyl formate	0.402	0.618
7. Chlorobenzene	0.417	0.714
9. Ethyl ether	0.423	0.667
10. Benzene	0.441	0.711
11. Carbon tetrachloride	0.452	0.742
Class 4		
12. Oxygen	0.497	0.872
13. Nitrogen	0.514	0.927
Class 5		
14. Mercury	0.636	1.41

As might be expected, as the temperature rises the values of e/λ for the unsymmetrical, come closer and closer to those for the symmetrical molecules, since as the temperature rises the increase in the energy of vibration of the molecules tends to decrease the extent of the orientation.

These relations are shown plainly in Fig. 10 and Table 2.

Energy of Thermal Emission

The energy of thermal emission (j) for a molecule is the mean energy transformed in moving it from the surface into the vapor, and is thus the heat of vaporization minus the total surface energy, or

$$j = \lambda - e$$

The value of the fraction j/λ is much more sensitive to changes of symmetry than that of e/λ. Thus at a corresponding temperature of 0.743 the energy of thermal emission for ethyl alcohol is four times that of surface formation, while for carbon tetrachloride the two have almost equal values. Table 3 presents the values of γ, l, e, j, and λ. with calculated values in parentheses. The values calculated for ethyl alcohol from its critical temperature involve the assumption that its molecules are symmetrical, which is known to be untrue. This table and the discussion which follows it are taken from a paper by Harkins and Roberts. The unit of energy used is the micri-erg, defined as 10^{-14} erg.

TABLE 3

Molecular Energy Values (in Micri-ergs) for the Vaporization of Liquids at a Corresponding Temperature Equal to 0.7

Liquid	T_c	γ	l	e	j	λ_i
1. Non-associated	Molecular = $\mathfrak{C}$					
Nitrogen	127	1.51	2.33	3.84	4.8	8.7
Oxygen	154.2	2.24	2.26	4.50	6.1	10.8
Ethyl ether	467.5	4.0	11.7	15.6	20.9	36.5
(Ethyl acetate)	(524)	(4.6)	(13.7)	(18.3)	(27.7)	(46.0)
Carbon tetrachloride	556	4.7	13.5	18.2	22.0	40.2
Benzene	561.5	4.8	13.7	18.4	23.3	41.7
Chlorobenzene	633	5.3	15.0	20.3	28.5	48.8
2. Associated						
Methyl alcohol	513	2.8	5.7	8.5	43.1	51.6
Ethyl alcohol	516.1	3.5	7.7	11.2	48.1	59.3
		(4.4)	(12.5)	(16.9)	(20.4)	(37.3)

"The data indicate that *at a definite corresponding temperature, in the case of non-associated liquids whose molecules are symmetrical, the molecular values for the latent heat of surface formation (l), the total surface energy (e), the energy of thermal emission (j), and the internal*

latent heat of vaporization (λ_1) *are nearly proportional to the critical temperatures of the liquids.* The same relation seems to hold for the free surface energy (γ) provided the temperature range is not too great. Thus the free surface energy of ethyl ether at a corresponding tem-

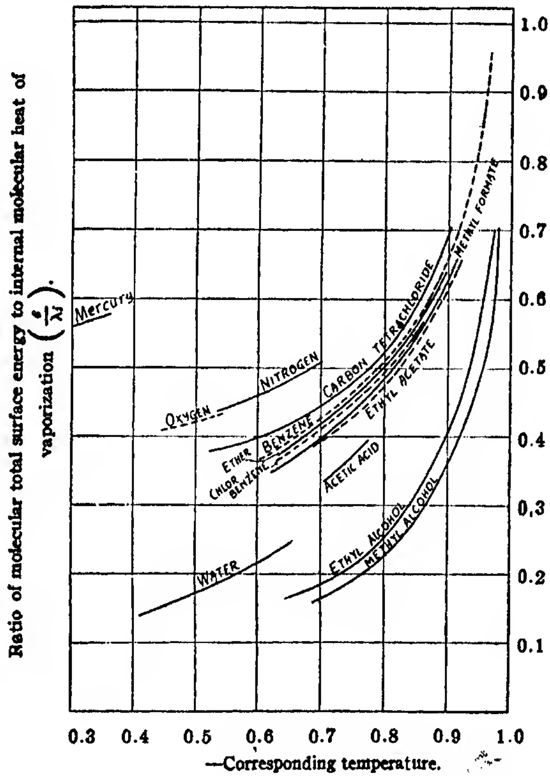

Fig. 10.

perature of 0.7 is 4.0 as calculated from the value of carbon tetrachloride, and 3.9, as calculated from the value for chlorobenzene, while the experimental value is 4.0. This statement as applied to the latent heat of vaporization alone, is somewhat similar to Trouton's law, which is known to be not entirely exact. Since the principle expressed above is much more general in its application, it is to be expected that it will prove to be somewhat less exact. That the energy values for

ether in Table 3 are lower than those for carbon tetrachloride is related to the lower critical temperature of the ether.

"2. *The effect of a lack of symmetry in the molecule, especially when marked, is to lower the molecular free surface energy, latent heat of surface formation, and total surface energy, and to increase the energy of thermal emission.* The values given in parentheses under those for

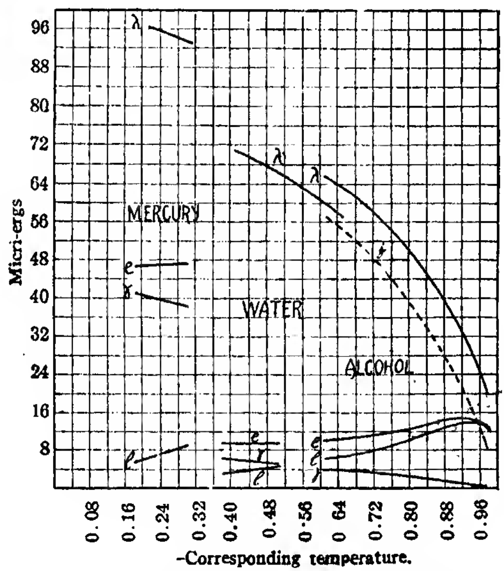

FIG. 11.—Values for the latent heat of vaporization (λ), latent heat of thermal emission (j), free energy of the surface (γ), latent heat of the surface (l), and total energy of the surface (e) calculated for a single molecule of alcohol, water, or mercury. It is seen that with the unsymmetrical molecules of alcohol both l and e exhibit a maximum at a corresponding temperature equal to about 0.90. In the case of symmetrical molecules, such as those of CCl_4 this maximum is absent.

ethyl alcohol, are those calculated from the critical temperature under the assumption of a symmetrical molecule, using the values for carbon tetrachloride as a basis. It is evident that the molecular free surface energy, and total surface energy, and more markedly the latent heat of surface formation, are considerably lowered by the dissymmetry of the molecule. The most striking effect is, however, the very great increase in the energy of thermal emission. The symmetry referred to in this discussion is that of the electromagnetic forces around the molecule, rather than a symmetry with respect to mass. The substitu-

tion of the slightly polar chlorine atom for hydrogen in benzene gives almost exactly the calculated value for a symmetrical molecule except in the case of the thermal emission (j), which is considerably increased, since it is the most sensitive of all of the quantities to changes of molecular symmetry. Since e is decreased, and j increased by increasing dissymmetry of the molecule, the ratio e/j serves as a remarkably sensitive index of molecular symmetry. This is illustrated in Table 3. The related ratio e/λ, which is equal to $\frac{e}{e+j}$, varies in the same way, but not so greatly.

"3. According to 'Stefan's law' the ratio of the total energy necessary to carry a molecule from the interior of a liquid into the surface to its total heat or energy of vaporization (e/λ) is equal to ½. That this is not the case is easily seen by a reference to Table 3, and Fig. 10. Not only is this an increasing function with increasing symmetry of the molecule, but also with increasing corresponding temperature. Its value seems to approach unity as the corresponding temperature approaches unity. Thus a molecule in the surface at a high corresponding temperature is, in terms of relative energy, very much more nearly in the vapor phase than when the corresponding temperature is low.

"4. The relations discussed in Paragraphs 1 and 2 above are just those indicated by the theory that molecules in the surface are oriented, the orientation increasing with increasing dissymmetry, and decreasing with increasing thermal agitation of the molecules. The effect of thermal agitation is illustrated in the case of the alcohols; compounds of the polar-nonpolar type. Fig. 11 indicates that for these compounds the molecular surface energy increases with the temperature. The effect of increased agitation is to overcome the orientation partly, and to throw the more polar groups into the outer surface, thus increasing the surface energy.

Fig. 11 gives the variation with the temperature of γ, l, e, and λ for mercury, water, and ethyl alcohol. The dotted line gives j for the alcohol.

Adsorption and the Distribution of Substances Between Regions

The adsorption of a substance at a liquid-vapor or a liquid-liquid interface may be treated as a special case of a solubility or a distribution problem. A region may be defined as a phase or an interface. Thus if a beaker of water is considered without taking into account what the beaker rests upon, there are the three phases: glass, water, and air (plus vapor), and the three interfaces: glass-air, glass-water, water-air, or six regions in all. If a fourth phase, benzol, is added, there are the following possible additional interfaces: benzol-glass,

benzol-water, and benzol-air, or ten regions in all. If a small amount of butyric acid is now added it will distribute itself between the four phases and the six other regions, with a different concentration in each. Thus it may be said that there are ten different solubilities for butyric acid in this system, that in the glass being the smallest of all.

Since the above system would require too lengthy a treatment, the glass and its interfaces with the other phases may be left out of the discussion. There remain the phases water, benzol, and vapor, and the interfaces water-benzol, water-vapor, and benzol-vapor, or six regions. This system has already been mentioned in an earlier section, but as a typical example it deserves more minute attention. The distribution which is attained at equilibrium depends both upon the temperature, upon the volumes of the phases and the areas of the interfaces, and also upon the amount of butyric acid added. At ordinary temperatures the concentration of butyric acid is lowest in the vapor. At low concentrations the solubility of the acid in the water is greater than that in the benzol, while at high concentrations the reverse is true. While this is undoubtedly a problem of kinetics, it may be considered more simply by considering the state in either phase at any instant. At low concentrations the association of the butyric acid in the benzol is small. At high concentrations, however, many carboxyl groups meet each other, as the result of molecular motion, and since their mutual attraction is greater than that for the benzol, they remain together a longer time than they would otherwise. Thus at any instant the number of double or triple molecules in the benzol is considerable. In the water, however, water molecules are themselves as polar as the carboxyl group, so the association is very much less since the carboxyl groups commonly attach themselves to water molecules instead of to other carboxyl groups. This may be expressed by the statement that single molecules of butyric acid are more soluble in water than in benzol, while double or triple molecules are more soluble in benzol. The solubility is greatest of all in the interface water-vapor, and next greatest in the interface water-benzol.

If we consider the region in which the solubility of the butyric acid is the greatest, it may be said to be the region which holds the acid with the greatest restraining force, or it is the best trap for the acid. Thus the best trap for butyric acid is the interface water-vapor, and the poorest is the vapor.

The greatest of all solubility rules is that like dissolves like, wherein the likeness is that of the electromagnetic stray fields (presumably largely electrical) around the molecules. Various methods may be used for determining the relative intensities of these fields for different substances. The following list has been drawn up by the writer from

a consideration of the surface tension relations, and is as follows, beginning with "those[4] substances around whose molecules the stray field is weakest; helium, neon, hydrogen (molecular, not atomic), argon, krypton, xenon, nitrogen, oxygen, methane, carbon monoxide, and the following organic compounds: saturated aliphatic hydrocarbons, aromatic hydrocarbons, sulfides, mercaptans, halogen derivatives (methyl chloride, carbon tetrachloride, chloroform, and ethylene chloride, with rapidly increasing fields) unsaturated hydrocarbons, ethers, esters, nitro compounds, nitriles, aldehydes, ketones, alcohols, amines, acids, and unsaturated acids. Following these are water, molten salts, heavy metals, boron and carbon. The list of organic substances is arranged for derivatives with short hydrocarbon chains. A lengthening of the chain causes a displacement in the direction of lower intensity for polar derivatives, but probably toward higher intensity in the case of the hydrocarbons themselves. It will be seen that in general the greater the distance between the substances in this list, the less their solubility in each other, the closer together the more soluble. For organic substances, though the present list is much more extensive, it is in agreement with that found by Rothmund from solubility data.[5] It is well known that metals in general give concentrated solutions only with metals, carbon (hydrogen), and other similar substances; molten salts dissolve salts or water; organic liquids are miscible unless at the very extremes of the list of organic substances; water dissolves salts or organic substances which are close to it in the list. An interesting illustration of this relation is given by data on the organic halogen derivatives listed above. The solubility of carbon tetrachloride per 1000 g. of water is 0.0052 mols, while that of chloroform which lies closer to water, is 0.068; and methylene chloride, approaching water still more closely, has a solubility of 0.236. This is also the order of increasing hydrogen content of the molecule, but that this is not the determining factor is indicated by the fact that methyl chloride and methane, similar compounds containing still more hydrogen, are much less soluble in water. In organic compounds the intensity of the stray field is much higher adjacent to what are commonly called double bonds, than it is near single bonds, and this intensity grows much larger still if triple bonds are introduced. Coresponding to this the solubility of ethane with its single bond between two carbon atoms, is 0.0507 volume of gas per volume of liquid; that of ethylene with its double bond is 0.1311, or more than twice as great, while acetylene with its triple bond has a solubility of 1.105, or about 22 times more than that of the single bonded compound."

In analyzing a solubility problem it is well to consider the attraction between the molecules of (A), between those of (B), and also that

[4] Quotation from a paper by Harkins and King, *J. Am. Chem. Soc.*, 41, 970-92 (1919).
[5] Rothmund, *Löslichkeit und Löslichkeitsbeeinflussigung*, Leipzig (1907), p. 118.

between (A) and (B). Consider octane and water which are mutually insoluble. It has sometimes been considered that this insolubility is due to the fact that water molecules attract each other more than they do molecules of octane, and that octane molecules attract each other more than they do molecules of water. Now the work of this laboratory shows that while the molecules of water do attract each other much more than those of octane, on the other hand the molecules of octane attract those of water very slightly more than they do those of octane. The much greater attraction of the water molecules for each other is a sufficient cause to produce immiscibility, since it is only necessary that when a group of water molecules is once formed the mutual attraction shall be great enough to cause the molecules of water to leave the group less often than they enter it so long as there is an appreciable quantity of water in the octane. The octane molecules are thus left in a phase by themselves.

In applying these ideas to surfaces it must be remembered that the stray field around a molecule falls off very rapidly as the distance increases.

While in applying the hypothesis, the intensity of the stray fields around the molecules is of primary importance, at least one additional principle must be used if the direction which any change will take by itself is to be predicted. As might be expected the second law of thermodynamics is of fundamental importance in this connection, and for this purpose it may be stated in the form: Any change which takes place by itself in a system will proceed in the direction which will result in a decrease in the free energy of the system. Thus a surface will decrease in area by itself, but will not increase. Since a rapid variation of the intensity of the stray field with the distance in any direction, is accompanied by a high concentration of free energy, the second law indicates that in any change which takes place by itself, the variation in the stray field becomes less abrupt. If we imagine the surface of a liquid up to a bounding surface plane, to have just the same structure as the interior of the liquid, then the actual surface always has a smaller free energy than would be given by calculation for this imaginary surface, and therefore the drop in intensity of the stray electromagnetic field at the actual surface is always less than it would be at a surface of the structure of the imaginary surface. Since a molecule is often made up of several species of atoms, the stray field around it is often unsymmetrical. Thus many organic molecules, such as the primary normal alcohols, acids, amines, nitro compounds, nitriles, ethylene and acetylene derivatives, etc., consist of a paraffin chain, around which the stray field has a relatively low intensity (a so-called nonpolar group), while at the other end of the molecule there is a group containing oxygen or nitrogen, sometimes with metals in addition, around which the intensity of the stray field is relatively high (a polar group). Such

molecules may be designated as *polar-nonpolar,* and designated by the symbol $\overset{\square}{\bigcirc}$ where $\bigcirc$ represents the polar, and $\square$ the nonpolar end of the molecule. If molecules of this type, such as butyric acid (C_3H_7COOH) are put in a two-phase system consisting of a polar liquid such as water, and a nonpolar liquid such as octane, then the free energy of the interface will be less when the transition from one liquid to the other is made by molecules of butyric acid, with their polar ends turned toward the water, and their nonpolar ends turned toward the octane, since in this way the abruptness of the transition is decreased.

The problem here arises as to the distribution of molecules of the polar-nonpolar type between the two liquid phases and the interface between them. It may be considered that each region (phase, surface, or interface) exerts a certain restraining force upon (has affinity for) the solute molecules. Since at equilibrium the thermodynamic potential of the solute is the same in all the regions, it may be considered that the concentration of the solute (at equilibrium) in each phase, interface or surface, gives an index of the restraining force exerted by that region upon the solute molecules. Let us now assume that we have a number of exactly similar two-phase systems, each of which consists of equal volumes of a polar liquid, such as water, and a nonpolar liquid such as octane, with an interface of a definite area between them, and into each of these systems we put N molecules of the polar-nonpolar type $\overset{\square}{\bigcirc}$. The hypothesis indicates that with a given polar group the distribution of the N molecules will vary in such a way that with an increase in the length of the nonpolar part of the molecule, the number of molecules, and therefore the restraining force in the octane, will increase, while in the water both of these will decrease. The reverse of this occurs when with a given nonpolar chain, there is an increase in the number of polar groups. The greatest restraining force would be exerted on such molecules when they are in the interface, where the nonpolar end of the molecules could turn toward the nonpolar liquid, and the polar end toward the polar liquid. Since the restraining force is greatest at the interface, the concentration in this region should also be the greatest, which agrees with the facts as found by experiment. This case is more complicated than when the volume phases alone are considered, so it will be discussed in a later section of the paper.

In the general case, where a component (A) is distributed between a number of phases (*a*), (*b*), (*c*), etc., the distribution will take place in such a way that when equilibrium is obtained the highest concentration of (A) will be found in the phase between whose molecules (or atoms if there is no formation of molecular aggregates) the intensity of the forces (and probably rate at which the intensity fall off with the dis-

tance) due to the electromagnetic field is most nearly similar to that around the molecules of (A). The least concentration of (A) will be found in the phase whose field is the most different from this, and in the other phases the concentration will increase as the properties of the phase approach those of (A) in the respect under consideration. The concentration in the various interfaces will be discussed in later paragraphs.

In the application of this very simple hypothesis certain complications must be kept in mind. The cohesion in nitrogen, for example, may be said to be due to the intermolecular field which is weak, and not to the interatomic field between the pairs of atoms, which is strong. In a like manner, if two butyric acid molecules unite to form a double molecule, the stray field is weakened, and the solubility in polar liquids such as water is greatly decreased, while that in slightly polar liquids such as octane, is greatly increased. The butyric acid is little associated in a dilute aqueous solution because most of its combinations will be made with the water (hydration) which is present in a much higher concentration than the acid. In extremely concentrated aqueous solutions, mostly butyric acid, the acid may be said to be associated to a considerable extent.

Relation at Surfaces and Interfaces

While at the surface of a solid or liquid the amount of gas adsorbed at a given gas pressure increases in general both with the cohesion of the solid or liquid, and also with that in the liquefied gas, provided the surface is not saturated, there are exceptions to this rule which may be explained on the basis of specific chemical reaction. That the above rule is in general true is indicated by many facts: The adsorption on a mercury surface is much greater than that on a water surface in the case of the ordinary gases which do not react with either surface to form molecular aggregates; and the adsorption on a carbon surface increases with the boiling point of the adsorbed gas, etc. Thus the general case may be considered from the standpoint of cohesion (or adhesion) and deductions obtained which are in accord with the facts.

To a certain extent either of the above methods may be used to advantage at the interface between two liquids, but in some respects it seems simpler to use the hypothesis in regard to the intensity of the stray electromagnetic fields, although all 3 theoretical methods of consideration are related to each other. If two phases, (A) with the higher and (B) with the lower intensity of stray field uniting the molecules, could be put in contact in such a way as to have the field of each perfectly uniform up to a plane phase boundary or interface between them, then the whole drop in intensity between the two phases would occur in a surface of infinitesimal thickness; so, at least by certain methods of mathematical analysis, the free surface energy would be infinite.

While this is not an actual case it suggests the idea that as the thickness of the transition layer increases the free surface energy diminishes. *With a given thickness of the surface layer the* free surface energy increases as the intensity of the stray field in (B) decreases, so the maximum free surface energy is reached when (B) is a vacuum, and is nearly realized when it is a dilute vapor or gas.

In so far as the cohesion of a liquid is an index of the average intensity of the stray field in a liquid it might be expected that the free surface energy between the given liquid A and a fluid phase B would thus increase as the cohesion in B decreases, *provided the thickness of the surface film remains constant.* The values of the cohesion which should be used in such a comparison are not, however, those for pure liquids, but should be the results obtained for the saturated solution of each liquid in the other—if equilibrium values are desired. It is manifestly true that the equilibrium value of the interfacial free energy between miscible liquids is always zero.

While a treatment of interfacial free energy from the standpoint of the relations of the average stray fields or of the related cohesion, is as satisfactory as is usual when problems, to a considerable extent molecular are treated from the standpoint of large scale phenomena, a more satisfactory theory may be obtained by considering at the same time, the molecular and atomic structure of the interface. An earlier paper from this laboratory [6] shows that when the Liquid A is water, and the Liquid B consists of long chain molecules, one end of each of which consists of a hydrocarbon chain, around which the intensity of the stray field is low, and the other end contains oxygen or nitrogen atoms, or double or triple bonds, around which the intensity of the stray field is relatively high, the free interfacial energy is low.

The transition from one electromagnetic stray field to the other would be made more gradual by a setting of the oxygen or the active atoms toward the water, and the hydrocarbon chains toward the benzene. The orientation in this case could not be expected to be perfect (in an undirectional sense) because two effects oppose a setting in the specified manner: (1) *The motion of the molecules in all cases partly prevents the orientation from being absolute,* so the orientation is a function of the temperature; it decreases as the temperature rises, and wholly disappears at the critical temperature; and (2) liquids of the type of B contain active (or polar) atoms, which also have a considerable attraction for the polar atoms of the surface film. The orientation is more complete at surfaces than at interfaces.

Adsorption at Interfaces

A two-phase system consisting of the phases (*a*) and (*b*) with a constituent (C) distributed between them, will now be considered. If the

[6] *J. Am. Chem. Soc.*, 39, 854-64 (1917), especially Table 1, pp. 856-7.

phase (*a*) consists of the vapor of (B′) mixed with the vapor of (C), where (B′) is taken as the only component of (*b*) aside from (C), then there are only two components. If (*a*) is a liquid consisting of a component (A) then there are 3 components. The drop in intensity between the two stray fields is greater in the former case, since the intensity of the field in a dilute vapor is extremely low. These two cases are illustrated by Fig. 12, where the ordinates represent the average intensity of the stray fields in the different cases.

The drop in intensity is more rapid at the interface water-vapor, since the interfacial film is of practically the same thickness in both cases.

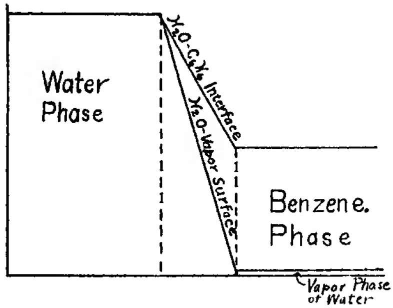

FIG. 12.

If the third component (C) consists of molecules of the polar-nonpolar type (□○) and is present in both phases, then by diffusion these molecules will pass into the interface, and when present in the interface in a sufficient number, will thicken the film and so decrease the free interfacial energy.

The drop in the intensity of the stray field shown in Fig. 12 may be considered as a restraining and an orienting force, similar to that existing in the field in air between the north and south poles of a magnet; and the molecules which are oriented as similar to small magnetized needles between them. The restraining force on the needles increases as the rate of fall of the magnetic intensity increases (increase of magnetic flux, or in the number of lines of force), so the restraining force holding the molecules might be expected to increase with the total drop of intensity of the stray field between the two phases, since the molecules of (C) in the interface bridge the total distance between the two phases.

If the molecules of (C) are thus held in the interface by a restraining force the molar activity or the molar fugacity (molar escaping tendency) of (C) will be much less in the interface than in either of the two-volume phases, so that the concentration of (C) in the interface

must be much greater than that in either of the volume phases to give a condition of equilibrium.

Since the fall of intensity at the interface water-vapor is much greater than that between water and benzene, the restraining force is much greater in the former case, so the average activity of the molecules of (C) will be much less at the water-vapor interface; and therefore to give equilibrium, the diffusion pressure of (C), in the two phases *water-vapor* will be much less to give a certain concentration of (C) in the interfacial film, than when the two phases are *water-benzene*. This is true so long as the thickness of (C) does not increase beyond that of a monomolecular layer. The present investigation shows that the facts are in accord with the above theory in cases so far investigated.

Now it happens that tightly packed monomolecular films of butyric acid with 2.77×10^{14} molecules per square cm. are formed either between water and vapor, or between water and benzol. At first sight this fact would seem to disagree with the theory developed above, which indicates that the interface water-vapor should be much the better trap of the two. However, that the facts are exactly in accord with the theory is seen when it is considered that the population in the traps depends both on the quality of the traps and upon the number of molecules to be trapped. Thus we find that to form the above monomolecular layer the number of molecules in the aqueous phase must be four times as great under the interface water-benzol as under that of water-vapor. In this sense the surface of the water is proved to be four times better as a trap than the liquid-liquid phase boundary.

The above determinations of adsorption were made by the use of the Gibb's adsorption formula

$$\mu = \frac{1}{RT}\left(\frac{\delta\gamma}{\delta \ln C}\right) T,$$

which has been proved by the work of this laboratory to give determinations of the number of molecules which agree fully with those obtained by direct measurement of the area of the films. Thus in the following table the number of molecules per sq. cm. for the first nine compounds was obtained in this manner by Harkins, King, and Clark, while for the last six compounds the results are those of Langmuir as obtained by a measurement of the area of the film. As the number of carbon atoms increases up to 16 the number of molecules per unit area increases, and beyond this number falls again. Evidently the two methods give practically identical results.

The present paper will be closed with a number of short statements concerning the fundamental principles of surface theory, together with statements of a few important facts.

1. The molecules in the surfaces of liquids seem to be oriented, and in such a way that the least active or least polar groups are oriented

toward the vapor phase. The general law for surfaces seems to be as follows: *If we suppose the structure of the surface of a liquid to be at first the same as that of the interior of the liquid, then the actual surface is always formed by the orientation of the least active portion of the molecule toward the vapor phase,* AND AT ANY SURFACE OR INTERFACE THE CHANGE WHICH OCCURS IS SUCH AS TO MAKE THE TRANSITION TO THE ADJACENT PHASE LESS ABRUPT. This last statement expresses a general law, of which the adsorption law is only a special case. If

TABLE 4

NUMBER OF MOLECULES IN THE "MONOMOLECULAR FILM" ON A WATER SURFACE[1, 2]

Substance	No. atoms carbon	No. molecules per sq. cm. $\times 10^{-14}$
1. Formic acid	1	(1.7)
2. Acetic acid	2	(2.0)
3. Propionic acid	3	2.6
4. Butyric acid	4	2.8
5. Valeric acid	5	(3.1)
6. Caproic acid	6	(3.2)
7. Heptylic acid	7	2.9
8. Nonylic acid	9	3.1
9. Decylic acid	10	3.3
10. Palmitic acid	16	4.8
11. Stearic acid	18	4.6
12. Cerotic acid	25	4.0
13. Octyl alcohol	8	2.9
14. Myricyl alcohol	30	3.7
15. Propyl formate	4	3.7

the molecules are monatomic, and symmetrical, then the orientation will consist in a displacement of the electromagnetic fields of the atom.

This law if applied to special cases indicates for a few pure liquids the following orientation: In water the hydrogen atoms turn toward the vapor phase and the oxygen atoms toward the liquid. With organic paraffin derivatives the CH_3 groups turn outward, and the more active groups, such as NO_2, CN, COOH, COOM, COOR, NH_2, $NHCH_3$, NCS, COR, CHO, I, OH, or groups which contain N, S, O, I, or double bonds, turn toward the interior of the liquid.

If any of these organic compounds are dissolved in water, their orientation in the water surface is the same as that just given, with the active groups inward. Table IV should be considered as part of this summary.

At interfaces between two pure liquids the molecules turn so that their *like* parts come together in conformity with the general law. With solutions, the solute molecules orient so that the ends of the molecules

[1] The results listed for Compounds 10 to 15 in Table I, were obtained by Langmuir, *J. Am. Chem. Soc.*, 39, 1848-1906 (1917).

[2] The results for Compounds 1 to 9 were obtained by Harkins, Clark and King in the years 1916 to 1919.

toward the liquid A are as much like A as possible, and the ends toward B are as much like B as possible. So at interfaces between organic liquids and water, for example, the organic radical sets toward the organic liquid, and the polar group toward the water.

2. If at an interface the transition from a liquid A to the liquid B is made by a saturated film of solute molecules which we may call A-B, that is, they have one end like A and the other like B, then the free surface energy is greatly reduced. For example, with water and benzene with sodium oleate as the solute, the free energy falls as low as 2 ergs per cm^2.

3. If the solvent is polar, such as water, then solutes will in general be positively adsorbed in the surface if they are less polar than water, and the least polar end of the molecule will be turned outward. Solutes more polar than water are negatively adsorbed.

4. The stability of emulsoid particles seems to be brought about by orientation of molecules at the interface with the medium of dispersion. The best emulsifying agents, for example, have very long molecules, with a polar or active group at one end of the molecule. For the emulsoid particle to be stable, the molecules which make the transition from the interior of the drop to the dispersion medium, or the molecules of the "film" should fit the curvature of the drop, at least to some extent. Thus soaps with one hydrocarbon chain give oil in water, and those with two or more, water in oil, emulsions.

5. The lowest known interfacial tensions for non-miscible liquids have been obtained by the writer and his associates, Dr. Wm. Thomas, and Miss Zollmann. At 20° the interfacial tension between water and benzol is 35.0 dynes per cm. Thus if with this two phase system water-benzol the aqueous phase is made 0.1 N with sodium hydroxide and with sodium chloride, and the benzol phase 0.1 M. with oleic acid, the interfacial tension is reduced to less than 0.04 dyne, or to approximately one thousandth the initial value. Even with much more dilute solutions, aqueous phase 0.01 M. in sodium hydroxide and 0.1 M. in sodium chloride, and benzol phase 0.01 M. in oleic acid, the interfacial tension is extremely low (0.09 dyne/cm.).

6. At the interface between mercury and an organic liquid the presence of a polar group increases the adhesional work, and thus presumably the attraction between the phases at the interface, but bromine and iodine have much more pronounced effects. Thus it seems that bromine and iodine, as well as carboxyl, orient toward the mercury.

7. When sodium hydroxide is added to a 0.1 molal solution of sodium nonylate, a remarkable effect is produced. The sodium nonylate solution alone has a surface tension against air of 20 dynes per cm., the lowest surface tension known for an aqueous solution. This is increased very rapidly as sodium hydroxide is added, and when the concentration of the base is only 0.008 molal the surface tension is

increased to nearly 50 dynes per cm., presumably by repression of hydrolysis. Further addition of sodium hydroxide decreases the surface tension in a linear way up to 0.5 molal when plotted against the concentration of the base (Fig. 13).

8. If emulsions of hexane or stanolax are examined under the microscope it is found that the greatest number of drops have a diameter of about 1.5 microns, whether the emulsions are produced by sodium or by caesium oleate. The distribution curve has somewhat the form of the Maxwell distribution curve. However there seem to be more

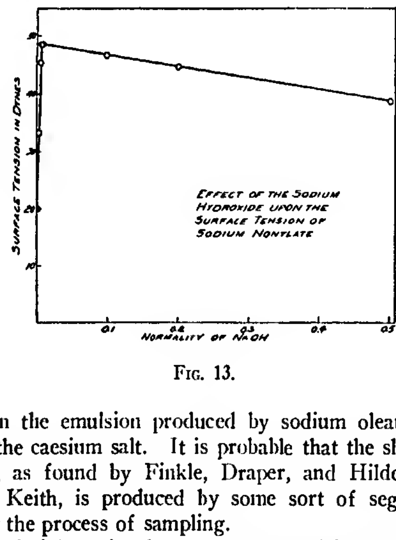

Fig. 13.

large drops in the emulsion produced by sodium oleate than in that produced by the caesium salt. It is probable that the shift in the peak of the curve, as found by Finkle, Draper, and Hildebrand, and by Harkins and Keith, is produced by some sort of segregation which occurs during the process of sampling.

9. The interfacial tension between water and benzene is 35.0 dynes per cm. The addition of sodium oleate to 0.01 molar concentration lowers the tension greatly to 2.29, or if 0.01 m. sodium hydroxide is present, to 2.16; and with 0.01 m. sodium chloride in addition, to 1.86. These values were obtained when the liquid phases were rotated until they came to equilibrium. For non-rotated solutions and 0.1 molar concentrations the surface tensions are: 2.64 for sodium oleate alone, and 1.23 if sodium hydroxide also is present. If 0.1 m. sodium hydroxide is present in the water, and 0.1 oleic acid in the benzene, the tension is 0.16, and with sodium chloride also in the aqueous phase it falls to 0.04, as cited in 5 above. These small interfacial tensions indicate that the soap reduces greatly the abruptness of the change in the molecular electromagnetic fields between water and benzene, particularly when the soap is formed in the surface by the chemical union

between the base in the aqueous phase and the acid in the benzene phase. Further experiments will be made to test the possibility that in such a system the chemical energy of the union of the acid and the base is transformed into molecular potential energy in the surface, thus lowering the amount of mechanical energy necessary to be supplied.

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THE SUPER CENTRIFUGE

The Effect of Super Centrifugal Force on Industrial Colloid Systems

By Lee H. Clark

Ten years ago the centrifuge was practically unknown outside of the dairy industry. To-day we can list well over one hundred and fifty specific applications of the Super Centrifuge in various lines of industrial work. Among the processes made possible by the use of the machine are many that I believe will be interesting to this meeting. It is my purpose to present some of the industrial accomplishments of the Super Centrifuge that seem pertinent to the study of colloidal systems.

To understand the work of a centrifuge it is convenient to consider briefly the phenomenon of gravity settling. If we allow a mixture of oil, water, and sand to stand undisturbed, gravity will tend to effect a separation into an upper layer of oil, an intermediate layer of water and a lower layer of the solid. Such subsidence is dependent fundamentally upon differences in the specific gravities of the constituents of the mixture. The familiar expression of Stokes' Law:

$$V = \frac{2r^2\ (S - S')\ g}{9\eta}$$

wherein

V = the velocity of settling
r = the radius of the suspended particle
S = the specific gravity of the suspended particle
S' = the specific gravity of the fluid medium
g = the gravity constant
η = the viscosity of the fluid medium

formulates the influence that the viscosity of the suspending medium, size of particle, and gravity differences play in determining the rate at which a separation occurs. Lacking a difference between the specific gravity of the suspending medium and that of the suspended material there is no tendency to settle.

The centrifuge effects the separation of solid particles and of liquid globules suspended in a liquid medium by centrifugal subsidence—centrifugal "settling." Centrifugal force is substituted for that of

gravity. As the direction of this force is outward from the axis of rotation of the centrifugal bowl, material heavier than the liquid medium tends to pass toward the periphery and to form a layer on the wall of the revolving bowl. Material lighter than the liquid tends to pass toward the axis and to form a column on the inner surface of the liquid.

A further consideration of Stokes' Law shows that as we substitute a higher force for that of gravity we increase the rate of settling directly as we increase the force. If experimental confirmation is necessary, the work of Svedberg and Nichols [1] on the determination of size of particles under centrifugal force affords it. A special centrifuge has been devised which allows measurement of the rate of settling of colloidal particles. By substitution of the observed rate in a form of Stokes' Law modified to allow for the known centrifugal force developed, the radius of the precipitating particle is calculated. Values obtained for r show nice agreement with measurements made with the ultramicroscope.

The increase in the rate of settling is but one of the functions of the Super Centrifuge. It is not so generally realized that centrifugal force actually effects separations impossible by an indefinite application of gravity.

Particles that are sufficiently subdivided to exhibit Brownian movement, tend to distribute themselves uniformly throughout a fluid. Opposing this tendency is the settling induced by gravity. Eventually a state of equilibrium is reached. According to Perrin's observations the concentration of the disperse phase increases in geometric progression with the algebraic decrease in the height of the level. Expressed symbolically this would be:

$$2.303 \log \frac{n_0}{n_h} = g.h$$

where n_0 is the concentration (number of particles per unit volume) in the initial level, o, n_h the concentration in the level h, and g the gravity constant.[2]

In many colloidal systems, the final distribution of the particles under gravity does not constitute a separation. From a practical viewpoint it is necessary to obtain the bulk of the continuous phase relatively free of the suspended particles, and the suspended material concentrated in but a fraction of the continuous phase.

Since the distribution of particles is dependent upon gravity a higher force substituted for that of gravity will alter the equilibrium conditions of such a system. Under certain conditions, the redistribution may constitute (for all practical purposes) a complete separation. Ayres [3]

[1] *Jour. Amer. Chem. Soc.*, 45, 2910 (1923).
[2] Ostwald-Fischer, "Handbook of Colloid Chemistry," p. 207, 2nd Edition.
[3] The Effect of Centrifugal Force on Colloidal Solutions. Ninth Annual Meeting Amer. Inst. of Chem. Eng., 1917.

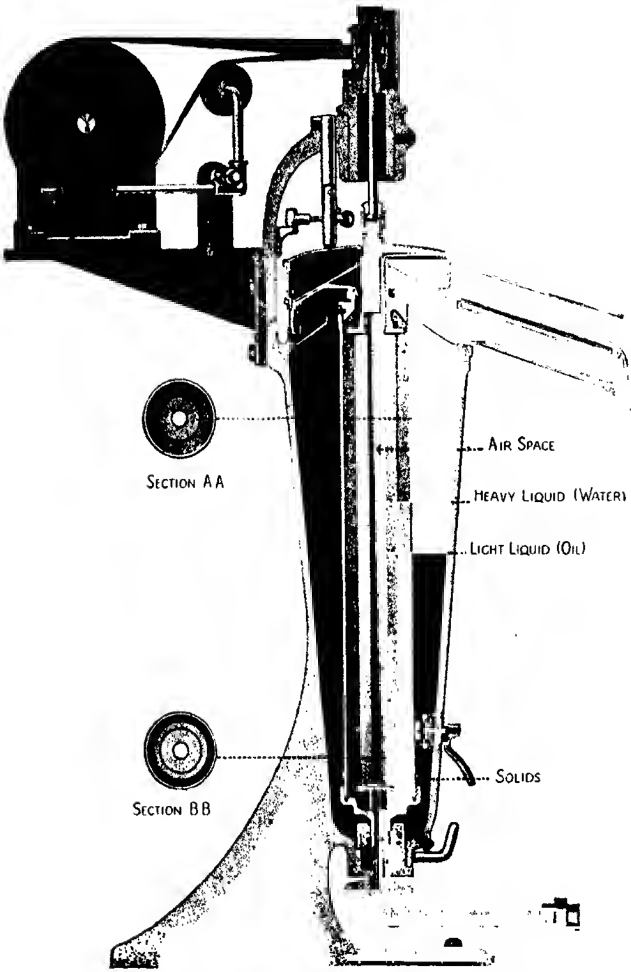

Fig. 1.—Sharples super-centrifuge. Sectional flow view.

has presented an interesting mathematical analysis of the problem. He finds that in a sol containing 1% of suspended solid a gravity separation will occur when the product of the effective mass of the particles (volume $\times$ difference between densities of particles and liquid) in grams by the constant 3×10^{16} is equal to 10. When this product is

less than 10 a partial subsidence will occur until an equilibrium is established.

By the application of centrifugal force, separation is complete when the product of the effective mass in grams, the force in dynes and the constant 3×10^{13} is greater than 10. Here again a partial subsidence will occur when the product is less than 10.

The time required to establish this equilibrium may be computed from a modification of Stokes' Law for velocity.

To take a definite example, silver particles in water will settle completely from a layer 1 cm. in depth in 12 days if they are larger than 30 $\mu\mu$ radius. For particles below this magnitude the equilibrium that is established does not constitute a separation. A force 50,000 times that of gravity (roughly the centrifugal force of the laboratory Super Centrifuge) will separate all particles of a silver/water suspension above .8 $\mu\mu$ radius in 5 hours time. Below .8 $\mu\mu$ the particles establish an equilibrium but stay suspended. These figures are also for settling in a 1 cm. layer (approximately the layer of liquid in the laboratory Super Centrifuge bowl).

It is a long step from a centrifugal separation requiring five hours time (necessarily a "batch" proposition) to commercial separations accomplished at the lowest rate in 6 minutes and at the highest rate in 6 seconds. An average time required for separations is 25 seconds. These figures represent the time a unit volume of liquid remains in the centrifugal bowl. The lowest rate corresponds to a flow of 15 gallons per hour, that used in handling viscous lacquers and cuprammonium cellulose solutions; the highest rate to 1000 gallons per hour reached occasionally in the dehydration of crude petroleum and in a few other cases.

The Super Centrifuge is an engineering answer to the demand for equipment to handle large volumes of liquids quickly and continuously, and to effect certain separations impossible by gravity methods. From what has been said above it is quite apparent that the higher the centrifugal force developed by a centrifugal that stays within engineering limits of safe and economical operation, the wider the range of problems over which that centrifugal may be used.

The commercial Super Centrifuge operates usually at 15,000 R.P.M., generating a separating force over 13,000 times that of gravity. The Sharples laboratory Super Centrifuge operates at a maximum of 40,000 R.P.M., generating a maximum separating force of over 42,000 times the force of gravity.

Many of the problems handled by these centrifugals are of interest from a colloidal viewpoint. Yet, were I to abide by definitions of the colloid state that limit it to systems having particles well below 1 μ in radius, I am afraid that I should have little of practical interest to discuss. In the laboratory, where the volume and time factor is of

secondary importance, suspensions having particles of such magnitude and below may be handled. The laboratory Super Centrifuge would seem to be an interesting medium for the study of systems, containing very finely divided particles.

In commercial practice, effective work is done on systems having particles usually of somewhat larger size. Yet in many respects they are of interest to the colloid chemist as they exhibit well defined colloid characteristics, in subsidence, in the effects of added electrolytes and in their formation or destruction by the proper colloids.

To obtain the most accurate conception of subsidence phenomena, it is well to avoid measuring results in terms of particle size alone. There are some emulsions (*i.e.*, water gas tar) with globules as large as one centimeter that exhibit distinctly colloidal characteristics. The globules in such emulsions, although huge in size, are of low effective mass, because the density difference between the specific gravity of the globule (water) and that of the medium (tar) is very small. Such small differences are, of course, uncommon. Low effective mass is most frequently caused by small volume. It is important, however, to bear in mind the influence of density in judging the work of the centrifugal. It explains the ability of the Super Centrifuge operating under commercial conditions to remove silver bromide particles as small as .5 μ radius from gelatin solutions, and its failure to separate cottonseed oil globules of 1 μ radius from soap stock emulsions.

It should not be inferred from what has gone before, that the usefulness of the Super Centrifuge is limited to a field in which it must compete solely with gravity settling methods. The Super Centrifuge is not a filter. It has been used, however, in fields at one time considered closed to the filter press. In filter press operation all liquid must pass through an accumulated press cake. Solids removed in the bowl of the Super Centrifuge are deposited against the walls and do not obstruct the passage of the liquid. The Super Centrifuge is therefore particularly adapted for use on viscous liquids and liquids containing gelatinous precipitates that make filtration difficult.

Before turning to a consideration of commercial centrifugal processes, I wish to call attention to one class of dispersoids that frequently settle by gravity within a short time, but are not removed completely in the centrifuge under normal operating conditions. In such cases, agglomeration occupies the larger portion of the time required for a gravity separation. An excellent example is ferric hydroxide in water. Unless substantial coalescence has occurred, such a precipitate, in passing to the centrifuge, is broken up into colloidal particles which are not removed completely. Comparison can be properly made between gravity and centrifugal force only when agglomeration is imperceptible.

Such problems are not entirely hopeless from a centrifugal viewpoint, however. To illustrate; at one stage in the manufacture of apple jelly

stock it is necessary to remove a voluminous, flocculent solid from suspension. In a typical case, gravity yields but 85% of clear liquid. The Super Centrifuge yields 98% of a slightly turbid liquid containing a very small amount of the deflocculated solid. Subsequent gravity settling of the centrifuged material is rapid and results in the recovery of all but 2-3% of a clear product. The liquid contained in this settled sludge is not lost as the sludge is returned to the following batch for centrifuging. The net result of the operation is the added recovery of approximately 13% of the total product.

This function of the Super Centrifuge to materially reduce the volume of a gel and so to recover liquids from loose flocculent sludges is of considerable importance where valuable liquids are handled.

Centrifugal operations may be classified conveniently under two general headings:

(1) The removal of solids from liquids, and

(2) The separation of liquids from liquids. In commercial operation, the materials handled have varied from those in which the dispersed phase is finely divided and well stabilized to systems that are relatively coarse mechanical mixtures.

The Removal of Finely Divided Solids from Liquids

The recovery of silver halides from waste photographic "emulsions" is, to my mind, one of the most interesting examples of the use of the Super Centrifuge for the removal of a finely divided, well stabilized solid from a liquid. These emulsions are formed by the precipitation of silver bromide in the presence of gelatin, followed by "cooking" to obtain the desired uniformity of grain. The smallest particles have a radius of about .5 μ. The particles of the emulsion remain very uniformly distributed even when the gelatine concentration is low and the suspension is maintained at a high temperature. They exhibit well defined Brownian movement in a dilute solution. The emulsion passes unchanged through ordinary filter paper.

The Super Centrifuge is being used commercially to recover the silver halides from waste emulsions, film cuttings and defective plates and films, incidental to the manufacture of photographic materials. Where it is desirable to recover the silver from used films and at the same time reclaim the film material, the emulsion is dissolved off and the resulting suspension centrifuged.

An application of a similar nature was the recovery of metallic platinum from the sulfuric acid "contact mass" left after the abandonment of the Government's powder plants at Old Hickory, Tennessee.[4] The mass, consisting mainly of magnesium sulfate carrying .2% of metallic platinum was dissolved to give a practically saturated solution.

[4] *Jour. Ind. & Eng. Chem.*, 14, 636 (1922).

The platinum was so subdivided that the bulk of it was unrecoverable by settling or filtration. It has been estimated that the recovery made by the Super Centrifuge was better than 99.5% of the metal that had been properly precipitated. In view of the haste with which it was necessary to carry on the operations, this may be considered an excellent showing.

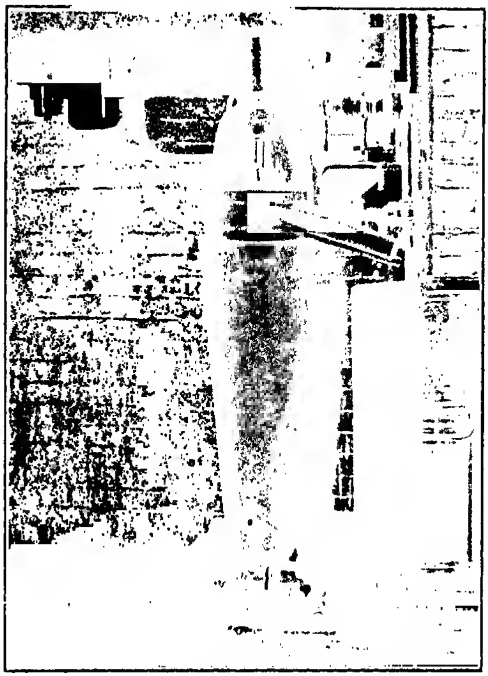

Fig. 2.—Super-centrifuge operating on the selective clarification of enamels.

The Super Centrifuge for "Selective Clarification"

Fractional centrifugation of sols has received some little mention in the literature. By this method Perrin[5] obtained uniform mastic and gambogue suspensions for his study of the distribution ratio of their particles. Uniform clay suspensions used by Svedberg and Nichols[6] were prepared with the Super Centrifuge.

It is only recently that the principle has achieved notable industrial

[5] "Brownian Movement and Molecular Reality."
[6] *Loc. cit.*

importance. Within the last two years centrifugal force has been widely used for the "selective clarification" of paints and enamels. The operation may be defined as the use of the continuous centrifuge for (1) the removal of larger particles of pigment without removing the smaller particles of the same pigment, (2) the removal of part or most of a heavier pigment without removing a lighter pigment, and (3) the removal of dirt and other foreign matter.

Briefly, the Super Centrifuge is to remove any coarse matter that may form visible specks to mar a smooth surface without removing the properly ground pigment.

To accomplish this result it may be necessary to control the intensity of the centrifugal force developed and to regulate the time during which the force acts on the suspension being centrifuged. The centrifugal force may be controlled by regulating the R.P.M. of the centrifugal bowl. Within certain limits fixed by mechanical arrangements for feeding liquid to the machine, the time factor may be controlled by the rate of flow of liquid to the machine. When it is desirable to obtain an even shorter period of treatment than is afforded by a standard centrifugal bowl, arrangements are made to regulate the amount of liquid the bowl will hold. By reducing the amount that can remain in the bowl, it is evident that the time of treatment of the liquid is correspondingly decreased, provided the rate of flow remains constant.

The range over which these factors are adjustable is very wide. Extremes of force are 520 to 13,000 times that of gravity (representing R.P.M. from 3,000 to 15,000); of flow rate 60 to 600 gallons per hour, and of liquid holding capacity of the bowl approximately zero to six quarts. Such variation in conditions means that there are sometimes more than one set of conditions to yield the same results.

To illustrate the principles of the operation, I have prepared a suspension of a mixture of carbon black and of lead chromate in linseed oil. The size of particles is approximately the same in both cases—.8 $\mu\mu$ radius. Their densities, of course, are very different. By centrifuging such a suspension at one half the normal speed of the machine and at a feeding rate of about 500 cc. per minute, the yellow chromate is almost entirely removed. An examination of the deposit formed in the bowl will show that this has been done without removing an appreciable amount of the carbon black. If the speed of the machine is raised to the normal 40,000 R.P.M. and the feeding rate reduced to about 200 cc. per minute, the removal of the carbon black is realized.

The Resolution of Emulsions

In separating a two-liquid system into its component parts, centrifugal force may have two functions, (1) subsidence, which serves to bring

the globules together, and (2) coalescence of the globules after contact. If we are dealing with an oil-in-water emulsion, subsidence will yield an emulsion, concentrated with respect to oil, and water, free or reasonably free of oil. If coalescence is obtained at the same time, oil and water will be obtained separately.

Centrifugal force is much better than gravity for subsidence in the majority of industrial cases. The size of the suspended globules is reasonably large and the rate of subsidence is directly proportional to the force applied.

In dealing with emulsions that are stabilized by the stronger emulsifying agents (*e.g.*, soaps and gums) centrifugal force under commercial

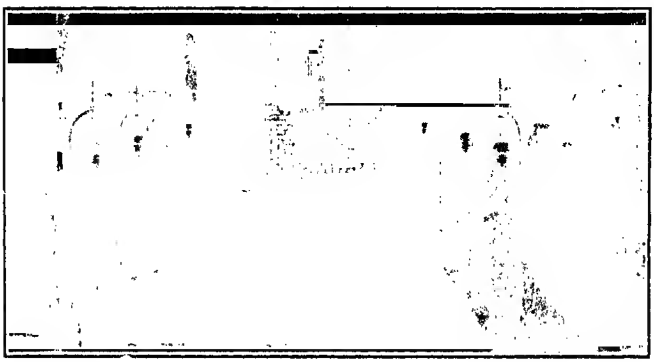

FIG. 3.—Battery of super-centrifuges for the recovery of vegetable oil from soap-stock emulsions.

conditions is but slightly, if any, better for coalescence than gravity. Small amounts of the proper reagent will do more to destroy or oppose the emulsifying colloid and thus cause coalescence, than an extended application of the highest commercial force.

Where emulsions are stabilized by solid particles centrifugal force is infinitely superior to gravity in causing coalescence. After a centrifugal separation, it will be found that the emulsifying agent has been removed from the interface between the liquids by subsidence. Contact of the globules is all that remains necessary to effect coalescence.

As far as I know, the phenomenon of coalescence under centrifugal force has never received mathematical or experimental study. It seems reasonable to suppose that the actual subsidence of the colloid particles of the emulsifying agent itself is, in most cases, necessary before coalescence can be obtained. In the case of the soaps, this particle may well be of molecular order, and tremendous forces would be necessary.

To cause substantial subsidence of a starch molecule (5 μμ) Ayres[7] has calculated that 1,000,000 times the force of gravity would be necessary.

The production of cream from milk is the most familiar example of the use of centrifugal separators for the concentration of an emulsion without coalescence of the dispersed phase.

Globules of butter fat in milk have an average radius of 1.5 μ. The dispersion medium is of low viscosity and gravity subsidence is relatively easy. To make a complete and rapid separation upon large volumes the centrifugal is necessary. The separation is carried out at a rate that is from 2 to 10 times as rapid as those used in other similar classes of commercial work. Yet a recovery is poor that drops below 99.5% of the available butter fat.

If we centrifuge milk at the higher force of the laboratory machine and allow a relatively long time for the force to act, a partial coalescence of the fat globules is obtained. At the same time a large proportion of the casein that stabilizes the emulsion is removed in the bowl of the machine. This lends added confirmation of the belief that subsidence of the emulsifying agent is necessary to coalescence.

The recovery of neutral oil directly from the stiff gelatinous mass of soap stock as produced in the refining of vegetable oils has not been possible centrifugally. To effect recovery, it is necessary to dilute with four parts of water. The resulting emulsion contains approximately 4% of soap and an equal amount of neutral oil in globules varying in size from .5 μ to 10 μ radius. There are, in addition to the soap, other constituents of the crude, namely vegetable resins and coloring matter which have distinct emulsifying properties in caustic solution. Such an emulsion shows very slight evidence of gravity subsidence.

At commercial rates, 60-75% of the emulsified oil is recovered centrifugally. All globules of 1 μ radius and above are removed and are discharged as a creamy emulsion containing at least 60% of oil. No coalescence results.

To obtain free oil from the emulsion recovered, a saturated sodium chloride brine is added. After the destruction of the soap film coalescence of the oil globules occurs, and the centrifuge is used to produce clean, dry oil from the mixture.

Some interesting data were obtained during the operation of the process. It was found that, in plants using softened water for dilution, the yields of oil were sometimes very low. The calcium salts in hard water decreased emulsion stability.

Experiments made to increase the yield of oil indicated a distinct advantage in the use of a small amount of calcium chloride. However, when enough was added to reach a point at which the value of the increased recovery more than offset the cost of the reagent, so much

[7] Loc. cit.

calcium soap was formed and dissolved in the oil that it interfered seriously with the subsequent caustic refining of the recovered oil, preventing proper coalescence and subsidence of the soap stock. A second method that gave the most satisfactory laboratory results but required too careful control for plant operation, consisted in the addition of about one quarter of 1% of both the sodium and calcium chlorides. Neither were as successful alone in the same or in larger amounts. The function of the sodium salt was to increase the size of the soap particle without causing actual "salting out." This weakened the soap film around the globules. Thus the calcium soap had a weaker colloid to oppose and the emulsion stability of the system was materially lowered. The use of sufficient of either reagent to destroy the emulsifying power of the soap was of course prohibitive from a cost standpoint and impossible technically as the precipitated soap would enmesh all oil in an unworkable gelatinous mass.

In the recovery of wool grease from wool-scouring liquors, we find the one instance of the subsidence and coalescence of oil from a soap solution. The soap concentration is normally about 1%. Whereas the original emulsion is of the oil-in-water type, the oil discharged from the centrifuge is in the form of a water-in-oil emulsion. Reversal of the form of the emulsion, after contact of the globules by subsidence, is probably caused by the strong hydrophobe characteristics of the grease (lanolin) itself.

To demonstrate a case in which the Super Centrifuge causes coalescence where gravity is ineffective, I have chosen an emulsion taken from a scrubbing process for the removal of naphthalene from illuminating gas. The emulsion consists of 15% oil and 85% water. The emulsion is of the water-in-oil type, containing globules 2 μ to as large as 2 mm. 80% of the oil will rise to the top rapidly by gravity. The rest, 3% of the total mixture, remains in emulsion form holding the water suspended in a remarkably stable condition. The settled emulsion is not broken down when heated and will stand unchanged for an indefinite period. It was found to be practically unaltered by a force 1000 times that of gravity.

In the Super Centrifuge, however, separation is rapid and complete. It is quite likely that the ready coalescence obtained by high centrifugal force is due to the removal of the emulsifying agent from the liquid interface by subsidence. The exact nature of the emulsifying agent is not known but, from the origin of the sample, it is probably some very finely divided solid material. Its concentration in the bowl of the machine is very evident.

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THE EFFECT OF SURFACE ENERGY ON COLLOIDAL EQUILIBRIUM

By H. O. Halvorson and R. G. Green

We have come to use the term "colloidal state" because of a realization that the general and fundamental properties of colloids are dependent upon the magnitude of the masses of matter concerned. The general chemist expects in his work to see a continuous and rapid transition of matter from large solid masses directly to a state of molecular dispersion. The colloidal chemist concerns himself with that particular state of matter where it exists as particles which have dimensions at various places between the two extremes. To bring matter into this state, the colloidal chemist must have the ability to control conditions so that the transition will be incomplete, or to make the process slow and subject to arrest. In dealing with colloidal equilibrium we are confronted at the outset by two problems, the first concerning the fundamental factors involved in the transition of any extent whatsoever, and the second concerning the limiting of the transition. The transition of large masses of a solid to a state of molecular and ionic dispersion is a very extensive change, involving a tremendous increase in that part of the substance which we call surface and finally reaching a state of division where our ideas of continuity as applied to surfaces no longer hold. It would appear that in the complete transition not only does a great change take place in the state of the matter, but the forces concerned appear in various modes of action which we are usually wont to call distinct. In short, for the complete transition to dispersion, we can think of the total energy as being represented largely by mass energy, then by surface energy, and lastly, by those energies associated with true solutions. In any state all must be present, and their relative value will depend upon the magnitude of the aggregates.

Several attempts have been made to give a general theory applicable to all classes of colloids which would define conditions active in maintaining a substance in the colloidal state. It would appear that anything so broadly applicable would have to be one taking into account transitions of energies as well as transition of dimensions of matter, in that the limits of the colloidal realm are ill defined and merge imperceptibly into the other recognized states of matter, solid substance and molecular solution. The problem becomes somewhat simplified if attention is

confined to a more or less limited range in the colloidal realm. In this paper we shall discuss the rôle of surface energy in maintaining stable colloidal solutions and so will consider for our developments a range of magnitude in which surface energy will have a maximum value relative to other energies concerned. We should, of course, expect the surface energy to be most important in the finer dispersed states, but as we approach the realm of single molecules and ions, we apparently lose continuity of surface and no longer have two phases to deal with. By confining our problem to aggregates of considerable size so that we have continuity of surface and two phases present, we can consider that we have a large portion of the energy of the system existing as surface energy and of a kind which can be represented by the work necessary to increase the surface. It would appear that the range of particle magnitudes concerned with surface energy changes could extend far toward the maximum magnitudes of the colloidal realm, since while the mass energy here would be larger and the surface energy smaller, yet any coalescence or agglutination of particles would be associated with a large change in surface area, and so a large change in surface energy.

We will consider stability of colloidal solutions from the viewpoint of thermodynamics in a rather simple manner and arrive at factors of colloidal equilibrium which concern the distribution of surface energies. An adequate consideration of surface energy does not seem to be included in the available critical discussions of theories of colloidal stability. The effect of surface energy is usually indicated by stating that the force of surface tension acts to cause coalescence or agglutination of particles, or to quote from Burton,[1] "as a conclusion, in the present state of our knowledge of these solutions, we may make the statement that the existence of the colloidal particle is fundamentally due to an equilibrium maintained between the forces of surface tension, and repulsion due to the electric charges."

A great mass of experimental data does not allow us to neglect considering the importance of the electric charge of the particle in colloidal stability. Experimental work indicates that there is a potential difference between a particle and a surrounding medium. We apparently do not know that for two particles in contact there is any similar and comparable difference in potential maintained. If we are to believe that the potential difference between particle and liquid results from any peculiarities of a given liquid in contact with a given particle surface, it would be illogical to assume that any similar and equal potential difference exists between two particles in contact as this represents an entirely different system.

It appears to us that we can take a more general view of the effect of this potential difference and in a manner more useful as well as less objectionable. We may consider that whenever aggregation or dispersion

takes place spontaneously, a change must take place in the potential energy of the system. This change in potential energy will be accompanied by a change in the extent of the particle liquid interface and the particle-particle interface. If the electric charge is important in aggregation or dispersion, it must be represented in the energy changes. If the charge is located at the surface of the particle it will be concerned with changes in the extent of the interfaces present. We can therefore consider that the surface energy changes occurring with a change of state may include surface energy characterized by the term surface tension and surface energy characterized by a potential difference occurring at the interfaces.

Thus it would appear that for the general colloidal realm we can look upon surface energy as greatly predominating, and for considerable changes in aggregation, can refer total energy changes, with a change in state, to surface energy changes. We can say then that for changes in the degree of dispersion of matter in the colloidal state, surface energy changes are fundamentally concerned. We can also consider that any process including either a dispersion of mass, or aggregation of molecules which comes to a stable state in the colloidal realm must have a condition of equilibrium associated with the surface energy of the system.

We must first consider what combination of conditions is necessary for either aggregation or dispersion to take place. The fundamental principles which would appear to govern these changes have been definitely set forth by Fucks, and can be stated as follows:[1]

1. If the molecules of the liquid have a greater attraction for the particles than they have for other liquid molecules, or than the attraction of particle for particle, two particles that are together will be forced apart twice the distance over which the molecular forces act.

2. If the attraction of particle for particle or liquid for liquid is greater than the attraction of particle for liquid, two particles that come close enough together to be acted upon by these forces will be drawn together.

These laws appear to be perfectly valid, and while they form a partial explanation as to conditions that bring about dispersion or aggregation, they do not contain any explanation of the factors controlling the extent of the process and so the degree of dispersion or aggregation. We are to concern ourselves with determining what factors are fundamentally important in controlling states of aggregation and dispersion, and further, just what conditions determine stable states in colloidal solutions.

The principles underlying the problems discussed in this paper have had their development in the problems of thermodynamics, but they have not to our knowledge been set forth definitely in the literature concerning the equilibrium conditions of the colloidal state.

When a colloidal solution reaches a definite degree of dispersion and remains so, it must be in a state of equilibrium, a condition in which certain functions must be at a minimum,[2] the condition being stable in that for any change in state to occur, an increase in these functions would take place. If this were not the case, a further change would spontaneously occur. From the second law of thermodynamics we know that in any process

$$dS - \frac{dU + W}{T} \geqq 0$$

where U = Total energy of system
T = Temperature
S = Entropy
W = Work done on the system by the surroundings.

If we define $F = U - TS$ when F = free energy of system we can then say that at equilibrium for an isothermal reversible process $- W - (dF)_T = 0$.

If we further imagine that our system is isolated or that no external work is done upon the system by the surroundings then $W = 0$ and therefore $(dF)_T = 0$. In other words when a system is at equilibrium and no work is done on the system the free energy must be at its minimum value. If in any system therefore the free energy is changing with respect to any variable, at equilibrium the free energy comes to a minimum with respect to that variable and $\left[\frac{dF}{dQ}\right] = 0$. Our problem then is to find a relationship between the free energy of the system and the extent of dispersion. This will enable us to find the rate of change of free energy with extent of dispersion as a function of the distribution of the surface energies of the system. We can then find under what conditions equilibrium can exist by determining when this rate of change will be zero.

In attempting to evaluate the energies of a system as represented by a colloidal solution, it simplifies treatment to represent the interfaces as zones definitely marked off from either phase and concern ourselves with the average energy in these zones. It is to be pointed out that the subsequent deductions do not depend upon these simplifications, but may be arrived at in a more general and rigorous manner.

We will imagine our system built up as follows. Let us consider that each particle has a surface zone which is different from the interior of the particle and different from the solution or liquid surrounding it.

Part of this particle surface zone will be in contact with the liquid and part with a similar zone of another particle. These two parts will in general be different, and we shall assign to each a certain value of average free energy per unit mass. This free surface energy may be of several kinds. It may be largely that surface energy charac-

terized by the amount of work expended in bringing molecules from the interior to build up new surface. This is the specific surface energy ordinarily referred to in surface films. Its value will vary with adsorption of substances into the surface and also with the presence of electrolytes in the surrounding solution. Further, and very important, this energy is a function of the extent of the interfaces when one phase of the system is a solution. The specific free energy of the particle surface zone may also consist in part of surface energy which may be characterized by the work necessary to bring an electric charge into the zone, due to the fact that there is a potential difference between the particle and the solution, as has been proved by abundant experimental work. We are not concerned with theories as to the origin of this potential difference, be it either a phenomenon of ionization or

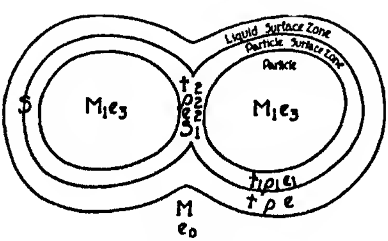

FIG. 1.—Colloidal particles in contact.

adsorption of ions. We are concerned only that a potential difference exists between the particle and the medium and that the charge is located on the surface of the particle. We do not need here to make the assumption that the demonstrated potential difference between the particle and liquid indicates a direct repulsive force between particles similar to that which would exist if the particles had static charges. The part of the total energy of the system due to this type of energy will vary with the extent of surface so that in considering dispersion and aggregation we will include this energy as part of the specific surface energy value assigned to the particle surface zone.

We will next consider that the liquid surface in contact with the particle surface zone also has a definite thickness at any point on the surface and can be represented by a liquid surface zone which will be different from the solution. This zone will be composed largely of the solution but may have in it substances in a concentration different from that of the liquid. The density of this zone may also be different from that of the solution. To this zone we will give a value of average specific energy and this may be different from that of the solution. This energy likewise will consist of energy characterized by work done in

bringing molecules from the interior of the phases into this surface and also by the work done in bringing an electric charge into the surface.

We may now proceed to indicate the various parts of our system by symbols to facilitate the equating of the total free energy to the free energy of the various parts. We will let various symbols have the following meaning:

M = Total mass of the solution.
M_1 = Entire mass of the particles.
$\Sigma t\rho$ = Mass of liquid per unit area in its surface zone where t is the thickness and ρ_1 is the density of the zone at any point.
$\Sigma t_1\rho_1$ = Mass of particles per unit area in its surface zone that is in contact with the liquid zone, when t_1 is the thickness and ρ_1 is the density at any point.
$\Sigma t_2\rho_2$ = Mass of particles per unit area in its surface zone that is not in contact with the liquid zone but with the surface of another particle.
S = Area of liquid particle interface.
S_1 = Area of particle-particle interface.
e_0 = Free energy per unit mass of liquid outside surface zone.
e = Average free energy per unit mass of liquid in its surface zone.
e_1 = Average free energy per unit mass of particle material in its surface zone in contact with liquid.
e_2 = Average free energy per unit mass of particle material in its surface zone in contact with a similar zone.
e_3 = Free energy per unit mass at interior of particle.
E = Total free energy of the system.

We may now equate the total free energy of the system, arriving at an equation similar to that derived by the authors for bacterial agglutination.[3]

$$E = (M - S\Sigma t\rho)e_0 + S\Sigma t\rho e + [M - S\Sigma t_1\rho_1 - S_1\Sigma t_2\rho_2]e_3 + S\Sigma t_1\rho_1 e_1 + S_1\Sigma t_2\rho_2 e_2.$$

This reduces to

$$E = (Me_0 + M_1e_3) + S[\Sigma t\rho(e - e_0) + \Sigma t_1\rho_1(e_1 - e_3)] + S_1\Sigma t_2\rho_2(e_2 - e_3).$$

Now as we are considering a system in which the surface energy is very large and the changes with which we are concerned involve primarily changes in surface, we will consider the mass energy constant and write

$$\text{Eq. 1.} \qquad E = M_E + S[\Sigma t\rho(e - e_0) + \Sigma t_1\rho_1(e_1 - e_3)] + S_1\Sigma t_2\rho_2(e_2 - e_3).$$

This equation states that the total free energy is composed of two parts, one part that is constant with the total mass, and another part that varies with the area of the interfaces, with the thickness and density of the surface zones, and with those quantities that represent the differences of the specific surface energies and mass energies.

Assuming that E, S, and S_1, are the only variables, we will differentiate the above equation.

Eq. 2. $dE = dS[\Sigma t\rho(e - e_0) + \Sigma t_1\rho_1(e_1 - e_3)] + dS_1\Sigma t_2\rho_2(e_2 - e_3)$.

We can simplify this equation if we can find a relation between dS and dS_1. Assuming that when particles come together or go apart there is no change in shape or volume of the particles, we can find such a relation. When S increases S_1 must decrease, and for every unit area of S_1 formed, two unit areas of S must have disappeared. Therefore $-2dS = dS_1$. Substituting this in our equation, we get $-[dE]_e = dS[2\Sigma t_2\rho_2(e_2 - e_3) - \Sigma t\rho(e - e_0) - \Sigma t_1\rho_1(e_1 - e_3)$.

If this process is carried out at constant temperature and volume we may write

Eq. 3. $$-\left[\frac{dE}{dS}\right]_{T,v,e} =$$

$$2\Sigma t_2\rho_2(e_2 - e_3) - [\Sigma t\rho(e - e_0) + \Sigma t_1\rho_1(e_1 - e_3)].$$

At equilibrium $-\left[\frac{dE}{dS}\right]_{T,v,e} = 0$. Therefore at equilibrium

Eq. 4. $$2\Sigma t_2\rho_2(e_2 - e_3) = \Sigma t\rho(e - e_0) + \Sigma t_1\rho_1(e_1 - e_3).$$

Equation 4 tells us the conditions that must exist at equilibrium. This equation enables us to discuss various factors of our problem.

1. It will enable us in any colloidal system such as we have imagined to predict the direction of any change that may occur, toward dispersion or toward aggregation.

2. It will allow us to draw deductions as to how this state of equilibrium may be changed.

3. It will enable us when used in conjunction with other thermodynamic relationships to show how any unstable system can ultimately reach a state of equilibrium. We will now consider each of these factors.

Suppose that our system is not at equilibrium. Let us see under what conditions this system will go to a state of more complete dispersion, and under what conditions the system will go to a state of greater aggregation. From Eq. 3 we can see that if (Eq. 5) $2\Sigma t_2\rho_2(e_2 - e_3) > \Sigma t\rho(e - e_0) + \Sigma t_1 e_1(e_1 - e_3)$ the coefficient of dS will be positive and then $\frac{dE}{dS}$ will be negative. Under these conditions, when S increases, E will decrease, and since the free energy will tend toward a minimum value, the system will spontaneously change to a state of more complete dispersion. If

Eq. 6. $$2\Sigma t_2\rho_2(e_2 - e_3) < \Sigma t\rho_2(e - e_0) + \Sigma t_1\rho_1(e_1 - e_3)$$

the coefficient of dS will be negative and $\frac{dE}{dS}$ will be positive. That is to say that the free energy will decrease with a decrease in S. The direction of this change will, therefore, be towards aggregation.

Let us now consider how a system in a state of equilibrium as expressed by Eq. 4 may be brought to an unstable state in which the inequalities as expressed by Eq. 5 or 6 will hold, and in consequence of which some change will take place with a decrease in free energy. If in a stable state changes are brought about in the specific energies so that e_2 is increased or that e or e_1 is decreased, Eq. 5 will express the resulting condition and greater dispersion will take place. If, however, e_2 is decreased or if e or e_1 are increased it can be seen that Eq. 6 will hold and greater aggregation will result.

We may now draw some deductions concerning the actual factors which we know are operative in colloidal solutions that may bring about these changes in state. In any solution the concentration of the various components may be different in the surface layers from that in the body of the solution. If we let a equal the excess number of molecules of a particular substance in the surface zone we know from Gibbs' deductions that $a = -\frac{c}{RT}\frac{d\gamma}{dC}$ where c is the concentration and γ the surface tension. This tells us that if we add a substance to a solution and the surface tension decreases, it follows that the substance concentrates in the surface layer, or conversely, if a substance concentrates in a surface layer, it must decrease the free surface energy of that layer. We may expect from well known experimental work on adsorption that if we have a system that contains various kinds of surfaces, any particular substance may be adsorbed in different degrees on these surfaces. In our system if we add some substance that will concentrate in the liquid surface zone or in the particle-liquid zone to a greater extent or with a greater effect than it does in a particle-particle zone it will bring about a state of more complete dispersion.

In a solution in which there are negative and positive charges, it is perfectly possible that the positive ions can concentrate in the one zone and the negative ions in the other decreasing thereby both e and e_1 This would result in a difference of potential between the colloidal particle and liquid and would also cause greater dispersion. It is also possible that certain ions from the colloidal particle may concentrate in the liquid surface zone and bring about the same effect. This would give us the well known phenomenon of charged particles in stable dispersion. We can see in a similar manner that if substances in the solution concentrate primarily in the particle-particle surface zone, it will decrease e_2 and bring about more marked aggregation.

In the Gibbs equation referred to above, a may be negative or positive.

It is possible therefore to add certain substances to our system that will increase e or e_1 and bring about aggregation or increase e_2 and bring about more marked dispersion. For these types of substances, however, the rate of change of surface energy with concentration is generally less marked than those referred to above and it will therefore take a much larger concentration of these substances to bring about the same degree of change in the inequality equations above.

We will now inquire as to conditions that may arrest changes in state before dispersion or coagulation is complete. It can easily be shown from thermodynamic considerations that the specific surface energy of a solution is a function of the surface area, that the surface tension of a solution changes with extent of surface. Further it can be shown that an increase in surface will result in an increase in surface tension in that $\frac{de_s}{dS}$ is always positive. We may apply this idea as the following proposition. If the distribution of surface free energies is such that a system is not stable and change in the extent of surface occurs, this change in surface area will bring about a change in the surface free energies as the process proceeds.

Let us suppose we have a system in equilibrium as expressed in Eq. 3 and consisting of large aggregates. We then must have the equality of terms expressed by Eq. 4. We may now imagine a factor added to the system that will increase e_2 and decrease both e and e_1. The inequality of Eq. 5 will now hold and the system will begin to disperse. As dispersion proceeds the interface S increases and the interface S_1 decreases. As the interface S increases, e and e_1 will increase, and as S_1 decreases, e_2 will decrease as $\frac{de_s}{dS}$ is always positive. We see that these changes occurring with dispersion are oppositely directed to the effect of the factor that brought about the unstable condition. If the original change in surface energies was not great or if $\frac{de_s}{dS}$ is of sufficient magnitude, as dispersion proceeds the equilibrium condition of Eq. 4 will again hold and a new stable equilibrium of greater dispersion will exist.

In a similar manner it can be shown that a system which is changing to a state of more marked agglomeration may be arrested before complete precipitation results.

In the foregoing deductions we have considered the free energy of the surface to consist of that energy covered by the ordinary concept of surface tension and also a certain free energy due to a potential difference between the surface of a particle and the surrounding liquid. This viewpoint enables us to see how it is possible to have aggregation or agglutination of particles that exhibit a charge and also how it is

possible to have a stable colloidal system in which the particles do not exhibit evidence of a charge. We then see that the effect of surface tension is not always to cause agglomeration of particles but under certain conditions actively maintains a dispersion.

It is to be pointed out that there are certain special cases of dispersion where there are factors to be considered in addition to those outlined above. As examples of such cases we may mention oil-water emulsions, where the relationship $-2dS = dS_1$, does not hold, and bacterial suspensions or blood cells in which the process of lysis is taking place. In this paper we have considered the effect of surface energies upon colloidal systems, considering changes in extent of surface only. It is probable that changes in the thickness and density of surface layers also occur, but consideration of these factors is not included in this paper.

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BACTERIA AS COLLOIDS

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The evolution of our Mother Earth from that vast, unenergized interjacence separating the heavenly bodies to the present day wealth of animal and vegetable forms of life is a chronicle of transcendental grandeur. It is a story of boundless space and limitless time. It seemingly begins with the awakening of the imponderable proto-matter, the so-called ether; the successive appearance of the gaseous hydrogen and helium stars; then the fiery suns, and the cooling planets. The physics of the nebula merges imperceptibly into the chemistry of the atom, the molecule, and the product of molecular reaction—the chemical compound—as the process goes on. A time comes when the statics of the chemical compound are merged into the dynamics of the colloid; mutable, plastic molecular complexes, in which the obstinacy of the chemical combination is replaced by a reactive, delicately poised equilibrium reminiscent in many of its properties of the phenomenon of primitive life. It is no mere coincidence, this labile, complex, molecular colloidal state, the height of inorganic evolution, and the unmistakable prominence of the colloid, in the protean manifestations of life in the scale of living things from the lowest even to the highest.

Between the lifeless colloid and the lowliest known living things there is a mental barrier: mankind as yet distinguishes utterly between the quick and the dead. Nevertheless, from the viewpoint of physical and chemical difference, the step from the complicated inorganic colloid to the simplest microbe, for example, seems shorter and more comprehensible than the passage from the unicellular, undifferentiated, asexual microbe to the vastly complex human being with its marvellous differentiation into synchronized chemical activities of unbelievable perfection, and the wonders of creative thought. The conception of the emergence of the quick from the dead involves a tradition of the human race that time alone can supplant.

It is not beyond the bounds of reason to look confidently to a day when science will triumph once again, and produce a colloidal matrix in which chemical families are enmeshed. This entire system seemingly will react along two chief lines: First, an imbibition or adsorption of certain substances, which are suitable both to enlarge the colloidal mass and to furnish something akin to food energy, from without the system, to enable it to carry on; and, secondly, to divide into at least

two fragments, each of which contains enough of the several chemical constituents to repeat the process, when the original colloidal mass reaches a size or state of inflation incompatible with continued enlargement. The driving force of this "bioidal complex" may well be either radio activity or the transformation of the energy of the sunlight into growth energy. In seeking for a photo-active element capable of bringing about such a transformation, one remembers instinctively that selenium is photo sensitive; and while it cannot be stated that selenium would bear the same relationship to the energizing of our hypothetical, primitive "bioid" that chlorophyll bears to the growth of the green plants of today, nevertheless, it is impressive to recall the potentialities of the chemical world at that time when our Mother Earth was ready for the advent of the great miracle, the appearance of life.

The bacteria appear to be the simplest of known living things as life exists at the present time. Biologically considered, they are extremely minute, rigid, unicellular organisms, without sex; they are devoid of chlorophyll or other photodynamic pigment; they have no morphologically demonstrable nucleus; and they divide by simple transverse fission, the resulting individual cells being of equal, or nearly equal size.

The absence of chlorophyll, or other photodynamic pigment, makes the bacteria dependent upon preformed, or organic food. This suggests that they are some distance removed phylogenetically from the probable primordial life, because the latter must have been independent of all substances of organic origin. The absence of a morphologically definable nucleus, however, makes bacterial multiplication a very simple process in comparison with almost all other known living things. Also, the asexual character of the bacteria removes the hereditary currents due to dual parentage. Therefore, after all, bacteria are close to the threshold of life—not so far removed in point of complexity from the most complex inorganic colloidal systems.

Chemically considered, the bacterial cell offers many important features for contemplation. The conception of bacteria as living chemical reagents has much to commend it. From this viewpoint, a microbe consists essentially of a colloidal matrix, or protoplasm, enclosed in a very delicate but extremely firm and rigid cell membrane. The two very essential vital phenomena of growth and multiplication are reduced to relatively simple processes in bacteria, when contrasted with corresponding phenomena among the higher organizations. Thus, microbic hunger is merely an influx of soluble substances of quite definite composition (discussed in detail later on), capable of furnishing structural and energy requirements for the colloido-chemical protoplasm of the cell; and microbic love is merely an expression of the inability of the enlarged colloidal complex to retain and absorb when it has reached a definite size, on the one hand, and of its ability to divide into two fragments, each retaining a share of all the parental chemistry, on the other

hand. In other words, microbic growth and multiplication are chemical phenomena involving action within, and the subsequent equi-partition of colloido-chemical families.

Before discussing in some detail the chemistry of microbic structure and microbic metabolism, and their dependence upon colloidal phenomena, it is essential to say a word about the size of bacteria because they illustrate in an exceptional manner the relationships between surface, volume (weight) and energy requirements of living things. Surface phenomena are greatly magnified with subdivision and bacteria are illustrative of this phenomenon most definitely. It is hardly necessary to point out the many exquisite illustrations of subdivision and their relation to chemical processes in the animal body; the red blood cells in an adult man some 27×10^{12} in number, have a combined surface area of some 3725 square metres, although their combined volume would scarcely fill a pint measure. They pass in rapid succession through the lungs, whose area of exposure to the air is estimated to be about ninety square metres. Each tiny red blood corpuscle carries but an infinitesimal amount of oxygen to the oxygen-hungry cells within the depth of the body, and yet the summation of their individual efforts is great indeed. Similarly, the surface of the intestinal tract is thrown into a multitude of minute folds which increase the absorptive surface relatively enormously without perceptibly increasing the bulk.

Similarly, bacterial cells are illustrative of this relationship between surface area and volume, and their well known ability to induce chemical changes in their environment, wholly disproportionate to their minuteness. The smallness of the bacteria, which will be commented upon at the proper time, must be associated with prodigious numbers to produce the necessary combined reactive surface area which their activities predicate. Bacteria may and do multiply with great rapidity. Thus, one of the most actively growing microbes is that one which causes, or incites, Asiatic cholera. Under the microscope the Cholera vibrio may be seen to divide into two daughter cells, conditions being favorable, every fifteen minutes for considerable periods of time.[1] The descendants of a single microbe, at the end of an hour, would be 16; at the end of two hours, at the same rate of growth, 256. In four hours the theoretical progeny would be 64,000; at the end of 24 hours—96 generations—a single germ would theoretically have given rise to no less than 8×10^{28} descendants. Of course this prodigious number is never realized. Nature interposes restraints and barriers, which keep the microbes within endurable limits, but nevertheless a man may be infected with cholera vibrios and die within twelve hours. Even though bacteria do not keep up such a rapid rate of multiplication as the theory would promise, nevertheless, millions of them develop within a comparatively few hours when conditions are favorable, and herein lies the numerical

[1] Kendall, "Civilization and the Microbe," 1923.

basis for the intensity of bacterial action. It should be remarked in passing that the amount of chemistry involved in that mysterious colloidal protoplasm of the microbe, to provide for such vast numbers of descendants, each a perfect replica of its kind, is great indeed. The definiteness and apparent readiness with which the multitude of simultaneous reactions take place in this colloidal matrix cannot fail to excite admiration and reverence for Nature's masterfulness.

The size of bacteria is the second factoı involved in their activities. A bacterial cell of average size, as, for example, the Cholera vibrio mentioned previously, measures about one mieron (0.001 millimetre) in diameter and two microns (0.002 millimetre) in length. Such a microbe would weigh very nearly 0.000,000,002 grams.[2] Its surface would cover an area of approximately 0.00001 square millimetres; the specific gravity is but slightly above that of water, hence, the ratio of surface area to weight of baeteria is nearly 1-2000. For comparison, a man 200 centimetres tall, weighing 75 kilograms, has a surface area of almost 2 square metres. The relation of surface area to weight in man is much nearer unity than it is in the microbe.

Inasmuch as the energy requirement of living things varies with the surface area rather than the weight, it will be seen that bacteria require proportionately much more energy than man. The influence of surface upon the intensity of chemical reaction—colloidal chemistry—is perhaps no more conspicuously shown than in the multiplicity and speed of reactions that take place side by side in the colloidal matrix of the microbic cell, with its concentrations at the interface between the interior of the extraordinarily thin semi-permeable membrane and the environment. This combination of an emulsoid protoplasm enclosed in a rigid semi-permeable membrane, while technically relegating bacteria to that class of colloids known as suspensoids, in reality causes the activities of bacteria to partake of the characters of each system.

The rigidity of the cell membrane and the turgor of the cell contents are great indeed. If the microbe is placed in a concentrated salt solution, the differentially rapid withdrawal of water results in a shrinkage of the cell substance, and the cell membrane may then be seen, often as a very delicate contour of the original organism; if the microbe be placed in distilled water, the colloidal protoplasm swells up and may burst the cell. The brunt of the shock in the microbic cell falls on the cell membrane when these violent changes in osmotic pressure are induced. Some possible conception of the forces at work may be surmised from the fact that approximately 2500 atmospheres of pressure are required to prevent the imbibition of water by starch.

At the cell membrane there is an interchange with the environment through which soluble and diffusible food substances pass in and soluble waste products pass out. Not only do diffusible substances penetrate

[2] Kendall, *Chem. and Metallurg. Engineering Journ.*, (1921), XXIV, pp. 56-60.

the membrane, but also lipoid-soluble substances as well seem to penetrate, due, according to Overton [3] and to Meyer [4] to their solubility in the lecithin and cholesterol content of the cell membrane.

The cell substance, protoplasm, is generally believed to be colloidal in character with various chemical families—as enzymes—enmeshed within it, but somehow kept sufficiently asunder to prevent confusion; and freely moving crystalloids. This is a veritable hive of industry in which oxidization and reductions, syntheses and analyses, and assimilation and excretion, occur with astonishing orderliness and marvellous precision. Apparently the colloidal protoplasm of the bacterial cell, unquestionably a complex substance containing the hereditary chemical architecture, is of unbelievable perfection. Not only is it able through its hereditary memory to recreate, almost without limit, its characteristic complex chemical structure; to elaborate within itself enzymes of exquisite definiteness, and to resist environmental pressure to change or modify its characters through periods of time; but also from one or another type of food substance it varies its percentage composition with reference to as basic an element as nitrogen, as the electrolytes and sources of energy vary. In other words, this remarkable colloidal matrix combines extreme specificity of chemical architecture with no discernible quantitative chemical formula for life.

The purely physical conditions for a multitude of quite distinct although dependent processes within the cell are admirable. The large surface area in proportion to the volume of the microbe provides for that enhancement of chemical interchange and reactions which is the basis for the observed magnitude of bacterial activity, in spite of their small size. The semi-permeable membrane regulates the ingress and egress of food substances, both for structure and energy, the elimination of waste products, and the retention of the complex chemical armamentarium which constitute the basis of life.

The colloidal contents of the cell, probably many quite distinct colloids —for enzymes are presumably colloidal—also keeps the various colloidal complexes within it apart, because colloids do not diffuse readily, thus insuring their independence of action without interfering with the diffusion of soluble and non-colloidal foodstuffs, and the products resulting from their excretion, into structure, or degradation with the liberation of energy. This makes possible a series of orderly transformations side by side, each process more or less dependent upon, and working normally in harmony with, the associated processes which comprise the chemistry of life. Chemical reactions can go to completion in the colloidal matrix of the cell with nearly the same speed as in water. The non-diffusible colloidal endo enzymes also are working most advantageously with the practical immobilization due to their colloidal

[3] Overton, *Jahrb. f. Wissenschaftl. Botanik*, (1900), XXXIV, 669.
[4] Meyer, *Arch. f. Exper. Path. u. Pharm.*, (1899), XLII, 109.

nature, the removal of products of their activity, either as more complex or less complex substances than the substrate upon which they act, and the delicate adjustment of reaction brought about by the phosphate buffer system, perhaps also with the bicarbonate, carbon dioxide buffers, described so carefully by Henderson.[5]

Even finely divided, nearly soluble substances may be acted upon in virtue of their great surface exposure in such a system. Thus, glycogen may be polymerized from glucose and stored as such until such time as the enzymic synthetic process reverses itself, and then the glycogen is depolymerized by hydrolytic cleavage and made available for energy.

Turning to the more detailed properties of the protoplasm of the cell, several features are well understood even though the great underlying problem of origin and specificity is wholly unsolved. The outstanding feature of the protoplasm from the physico chemical viewpoint is its asymmetry. It contains proteins, which constitute the hereditary chemical architecture of the cell, made up of amino acids tied together in the manner and in the order which constitutes this specific architecture, and this protein complex possesses in the aggregate a dual asymmetrical activity. It reforms itself, in obedience to the urge of growth, exactly upon its ancestral plan, weaving amino acids or their simple complexes into l-rotating specific proteins of like kind. In this respect plant cells differ from bacterial and animal cells, in that they utilize, through the chlorophyll of their leaves, solar energy, and can synthesize protein from mineralized nitrogen.

The other manifestations of protein symmetry are shown both in animal and bacterial cells (and so far as is known by living cells in general, which do not contain a photodynamic pigment or its equivalent) by the exquisite specificity of their protoplasm, or enzymes contained therein, for energy producing substances. Various sugar configurations —almost always d-configurations [6]—are so specifically used or rejected as sources of chemical energy that the cells manifesting this specificity can be used with definiteness as reagents for the identification of such substances. The delicacy of the reaction is greater than that of known chemical processes, and the fact that cells manifesting this specificity can, so far as known, make no mistakes, would seem to emphasize the amazing specificity of cellular activity.[7]

The chemistry of the asymmetric carbon atom in relation to cellular activity is shown in various other ways. Substances to be stored, as fats or starches (glycogen), in the animal body are either symmetric with reference to their carbon atoms—for example, fats,—or nearly insoluble polymers of reactive substances, as glycogen. This would seem to be a desirable method for removing the reserve foods from the field of immediate decomposition for energy.

[5] *Erg. d. Physiol.*, (1909), VIII, 254.
[6] Fischer says always d-configurations. *Ber. d. deut. chem. Gesell.* (1894), XXVII, 2031.
[7] Kendall, *Journ. Infect. Diseases*, (1923), XXXII, 362.

Many important physiological effects flow from this predilection of the asymmetric carbon atom for vital processes. In the first place, it is believed that symmetric substances may have a somewhat greater density than corresponding asymmetric substances of the same composition.[8] Structural vital constituents, as proteins, are commonly l-rotating, while energy-giving vital constituents are usually d-rotating. This is significant, in so far as the facts are known. Fischer[9] commented upon the relation of chemical asymmetry to the vital processes in no uncertain terms, and indeed referred to the entire field as "bio geometry," and fruitful for future exploration. Lest we get too far afield, it should be borne in mind that these instances are the outgrowth of vital activities apparently inseparable from colloid chemistry.

Referring more intimately to the relationship between optical rotation (asymmetry) and physiological characters, Piutti[10] claims that d-asparagin has a sweet taste, whereas the l-asparagin is without taste. Strangely enough, the corresponding d and l aspartic acids are alike insipid. In like manner, Menozzi and Affiani[11] states that d-glutaminic acid is sweet although the l-form is tasteless. Similar observations have been made in relation to odors, in certain instances. Here apparently there is a relationship, quantitative if not indeed qualitative, between certain asymmetric organic compounds and the induction of a physiological response.

Closely associated with the phenomena of chemical asymmetry and physiological response just mentioned is the relation between bacterial enzymes and their action upon proteins and carbohydrates.

Enzymes of microbic association are divided, for convenience, into endo or insoluble enzymes, enmeshed in the colloidal protoplasm of the bacterial cell and not passing without unless the cell membrane be ruptured, and the soluble or exo enzymes, which are thrown out of the cell and act upon complex proteins, carbohydrates, or their derivatives preparing them for assimilation within the cell.

It is worthy of note that the endo enzymes, which have to do largely with the anabolic or synthetic activities within the bacterial cell, are able to pass into a quiescent state in spore forming bacteria during the spore stage, and do not autolyze the contents of the cell at this time, although they seem to do so when non sporulating bacterial cells are placed in water, or aqueous solutions not containing the ingredients requisite for life. These endo enzymes, aside from the one or ones which are presumably concerned with the highly important function of transforming glucose or similar carbohydrate into energy, are hydrogenic, that is, they polymerize simpler sugars, they form fats and they synthesize the protein of the protoplasm for simpler substances, as

[8] Wallach, *Liebig's Annalen* (1895), CCLXXXVI, 185; Liebisch, *Ibid.*, p. 140; Walden, *Ber. d. deut. chem. Gesell.* (1896), XXIX, 1699.
[9] *Ber. d. deut. chem. Gesell.* (1894), XXVII, 3189.
[10] *Compt. rend.* (1886), CIII, 134.
[11] *Gazzetta* (1887), XVII, 126, 182.

amino acids or polypeptids by removing from the carboxyl group of one amino acid and the hydrogen of another amino acid hydrogen and oxygen in the proportions to form water. This is done with due reference to the intimate complex, and exquisitely specific arrangement in the latter instance, of the numbers and kinds of amino acids and their complexes which make up the hereditary chemical complex which constitutes the individuality of the microbe itself.

It is not definitely known whether or not the protoplasm of the microbe can transform one amino acid into another to make up a deficit in available and necessary ones for the protein architecture, but it is known that tubercle bacilli, for example, can construct at least a skeleton protein with all the heredity that goes with a tubercle bacillus, from a medium containing ammonium phosphate as the sole source of nitrogen in solution.[12] It should be stated that this primitive tubercle bacillus is morphologically, and in tinctorial properties quite unlike a tubercle bacillus growing under favorable conditions in the culture tube or the body, but such an atypical organism will resume its characteristic appearance and staining qualities when it is placed in a proper nutritive environment. Such a remarkable laboratory is this colloidal protoplasm of the microbic cell!

Turning to the exo enzymes of certain bacteria, as Bacillus proteus, which acts upon soluble proteins or their derivatives outside of the microbic cell, there is much evidence that these enzymes, unlike the hydrogenic enzymes in the cell, are not specific in their action; they are not formed in an active state if the energy needs of the microbe are supplied by utilizable carbohydrate.[13] In other words, the hydrolytic proteolytic enzymes of Bacillus proteus and related organisms are apparently produced solely in response to the energy needs of the cell.

The enzymes which act upon carbohydrates are endowed with an astonishing specificity. Fischer [14] discovered this high grade specificity in his classical studies upon yeasts, making use of his famous simile of the key and lock to explain the relationship. Pasteur,[15] however, in his studies of the action of the mold Penecillium upon the tartaric acids, seems to have been the first to appreciate and utilize the relation between the configuration of certain organic substances which occur naturally in the d and l forms, and the ability of the protoplasm of living organisms to use them. This striking specificity of protoplasm has been made use of to detect, determine and to identify sugars.[16] Very small amounts of carbohydrates may be identified and estimated quantitatively by these microbic reagents. In some instances, as for example glucose in the presence of lactose, bacteria have a great advantage over the instruments

[12] Kendall, Day and Walker, *Journ. Infect. Diseases* (1914), XV, 433.
[13] Kendall and Walker, *Journ. Infect. Diseases* (1915), XVII, 442.
[14] *Ber. d. deut. chem. Gesell.* (1894), XXVII, 3189.
[15] *Compt. rend.* (1858), XLVI, 615.
[16] Kendall and Yoshida, *Journ. Infect. Diseases* (1923), XXXII, 369.

of precision of the physicist or the reagents of the chemist.[17] Bacteria have no brains. They have no choice and, therefore, cannot of themselves make mistakes in such procedures.

One final example of the exquisite specificity of microbic action deserves mention. Certain bacteria, as the tetanus bacillus, which causes the dread infection known as lockjaw, form within their substance extremely potent poisons which are not only highly specific, each after its kind, but also equally specialized in that they act upon definite cellular systems. Thus, the tetanus poison, or toxin, acts solely upon the gray substance of the brain. It has no action upon other tissues. The brain substance of cold-blooded animals is not affected by this tetanus toxin. Apparently, the more highly differentiated protoplasm of the brain substance of the warm blooded animals alone contains the receptor for this toxin. This colloidal toxin, then, possesses a high degree of specificity for the colloidal protein substance of certain highly specialized cells of the mammalian host.

Examples might be cited and instances multiplied many times of this striking specificity of reaction products, not only of bacterial cells, but also of cells of the human body. Merely to cite adrenalin, or epinephrin as it is sometimes called, will suffice to bring a new group into contemplation—those of the glands of internal secretion which are regulators of cellular processes in remote parts of the organism. All of these phenomena are, so far as is known, wholly dependent upon and inseparably associated with colloidal chemical activity.

At the present time a mere recital of some of the outstanding features of the colloido-vital complex is about all current information warrants; science must look to the colloidal chemist for the unfolding of those intricate phenomena which collectively comprise the dynamics of life.

[17] Kendall, *Journ. Indust. and Engin. Chemistry* (1923), XV, 1001.

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THE EFFECT OF AMMONIUM SALTS UPON THE SWELLING OF COLLOIDS AND UPON THE GROWTH OF YEAST AT VARIOUS TEMPERATURES

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As a general thing fundamental principles are formulated in the course of research in so-called "pure" chemistry and then passed on to the worker dealing with the living cell. Occasionally the order is reversed and the generalization is made from the results of the investigation of the living protoplasmic unit. That this is possible and in the future more and more likely follows from the fact that a bacterial emulsion is a heterogeneous system and lends itself admirably to certain types of study. In the following communication the author hopes to be able to point out some interesting generalizations obtained during the study of factors concerned in the growth of yeast and to show their applicability to other heterogeneous systems.

In 1907 Pringsheim [1] published a paper in which he dealt at length with the effects of various nitrogenous materials upon the fermentative activity of several types of yeast. In several instances he found a so-called "optimum" concentration for the rate of fermentation but it is remarkable that in the case of ammonium sulphate with Frohberg yeast there were two maximal concentrations at 0.378 and 3.026 grams of salt per one hundred cubic centimeters of medium. Boas [2] confirmed the above finding and came to the conclusion that the phenomenon could be explained only on the basis of alteration in the permeability of the cell wall. At the maximal point the cell wall was "aufgelockert" while at the minimal point it was "verdichtet." He would class nutritive materials for microörganisms into the "membrane active" and the "membrane passive" categories. He presented no experimental data on the effect of the ammonium salt upon the permeability of the cell wall but reached his explanation by a process of elimination of the possible causes which he considered.

During the course of the development of a simple synthetic medium for the growth of yeast, Fulmer, Nelson and Sherwood [3] again noted the maximal concentration effect of ammonium salts and found that at 30° the four ammonium salts, the chloride, sulphate, nitrate and tartrate, promoted a maximum crop of yeast at the same normality, that is 0.0353N. The four salts at the above concentration exhibited no significant differences in yeast growth stimulation. That the phenome-

non cannot be due to hydrogen ion concentration is shown by the fact that the maximal normality of ammonium salt is the same with or without 0.1 grams of calcium chloride per 100 cc. of medium. The phenomenon then would seem to be a function of the ammonium ion concentration.

It was likewise pointed out that with rise in temperature there was required a higher concentration of salt for maximal growth and that the largest crops were obtained at 40° C., a temperature considerably higher than that usually considered to be optimum for the growth of the yeast. This at once suggests the name "thermal buffer" for the salt and indicates the importance of the composition of the medium upon the thermal death point of an organism or the efficiency of heat in sterilization. This should apply to thermal effects upon other reactions in heterogeneous systems.

In seeking for a possible explanation of the maximal effect of the ammonium salt it was found [3] that the optimal concentration for the growth of yeast for each temperature tested was identical with that in which a protein (wheat gluten) was least swollen. Since that time additional data have been obtained by Sherwood showing the two maximal points previously discussed, both points varying with the temperature and at the temperatures tested being the concentrations of the salt in which gluten was least swollen. A summary of such data obtained in this laboratory is given in Table I. In order to determine whether any general principle might be involved a parallel study was made of the stimulative effect of ammonium chloride upon the growth of yeast in beer wort and upon the swelling of gluten in wort ammonium chloride systems.[4] As with the synthetic medium (see Table I) there were two maxima which varied with temperature and which were identical with the concentrations of the salt in which gluten was least swollen. The fact that less of the salt is required for the maximum effect in the wort than in the pure medium shows that there is present normally in wort something which plays a similar rôle to that of ammonium ion. The generalization seems to be indicated that if the addition of an ammonium salt to a medium increase its ability to dehydrate gluten it will likewise improve the medium for the growth of yeast, a maximum growth of yeast being obtained in any such system in which the gluten is least swollen. The concentration of ammonium salt required for the maximal growth of yeast and minimal swelling of gluten increases with rise in temperature. A study of the data in Table I reveals the fact that the "critical" concentrations of the ammonium salt are a linear function of temperature. The equations to the straight lines are $a_1 = 0.00057t + 0.0179$ for the first maximum and $a_2 = 0.0042t + 0.111$ for the second. By means of these equations it is possible to calculate the normalities of ammonium chloride required for the maximal growth of yeast and the minimal swelling of gluten at any temperature.

TABLE I

NORMALITIES OF AMMONIUM CHLORIDE MAXIMAL FOR THE GROWTH OF YEAST (a) AND MINIMAL FOR THE SWELLING OF GLUTEN (b)

Temperature	I		II	
	(a)	(b)	(a)	(b)
0	0.0118	—	—	—
10	0.0236	—	0.1534	—
20	0.0295	0.0295	0.1940	—
25	0.0319	0.0319	—	—
30	0.0354	0.0354	0.2360	0.2360
30 (wort)	0.0236	0.0236	0.1060	0.1060
35	0.0383	0.0383	—	—
40	0.0413	0.0413	0.2780	0.2780
42	0.0425	0.0425	—	—
43	0.0431	—	—	—

$$a_1 = 0.00057t + 0.0179$$
$$a_2 = 0.0042t + 0.111$$

From the above the following generalizations seem to be indicated. Data obtained at one temperature regarding the effect of various reagents upon the swelling of colloids, catalytic activity, or other reactions in heterogeneous systems are not necessarily true for any other temperature. The so-called lyotropic series may not present the same order at every temperature. It seems likely that the distribution of dialyzable ions on the basis of the Donnan equilibrium and iso-electric point vary with temperature and studies of this sort are in progress.

It has been shown that for each temperature there are at least two critical concentrations of ammonium chloride. It follows that for each concentration of the salt there are two critical or optimal temperatures which can be calculated by means of the equations $t_1 = N - 0.0179/0.00057$ and $t_2 = N - 0.111/0.0042$, where $N =$ the normality of the salt. In Table III are listed several of the standard media which have been used for the growth of yeast and in which an ammonium salt is the only source of nitrogen. It has been found in

TABLE II

CALCULATED OPTIMAL TEMPERATURES FOR SEVERAL STANDARD MEDIA USED FOR YEAST GROWTH

Investigator	Ammonium Salt	Normality	Optimal	Temp.
Pasteur	Tartrate	0.00109	—29.5	—26.0
Nageli	Tartrate	0.00435	—23.8	—25.4
Mayer	Nitrate	0.00936	— 15.0	—24.2
Amand	Chloride	0.0187	+ 1.4	—21.9
Raulin	Nitrate and sulphate	0.0359	+31.6	—17.9
MacDonald	Sulphate	0.0454	+48.2	—15.5
Laurent	Sulphate	0.0713	+93.7	— 9.5
Cohn	Tartrate	0.109	+159.6	— 0.47

this laboratory that the concentrations of only the ammonium salt need be changed with temperature for a synthetic medium containing such salts and calcium chloride, di-potassium phosphate, and so forth. So at least approximate figures may be obtained for the optimal temperatures from the media listed. It is apparent that from only one of the media, Raulin's, does an optimal temperature fall within the viable range. It may be stated then that where ammonium salts are concerned, that there is the possibility of two optimal temperatures for the growth of microörganisms or for other reactions in heterogeneous systems.

The above considerations quite naturally lead to the study of temperature coefficients of reactions in heterogeneous systems at various temperatures. It is obvious that in the case of ammonium chloride with yeast and with the gluten an increase in temperature at a constant concentration of the salt is equivalent to the lowering of the concentration at constant temperature. If the temperature coefficient is to represent only the effect of temperature upon the rate of reaction, the concentrations of the materials in solution must be adjusted. This is easily illustrated by the effect of temperature upon the growth of yeast. If the medium contained the concentration of ammonium salt optimum for 1.8° C. (Amand Table III) and the relative growth rates obtained at 10° and 30° C. the rate will be accelerated by rise in temperature but retarded by the fact that the medium is becoming relatively poorer because increasing distance from the optimum. On the other hand with a medium optimum for 48.2° C. (MacDonald) the growth rate will be accelerated from 10-30° not only by temperature but because of the approach to the optimum condition. In the case of a medium like that of Laurent or Cohn the effect will be complex. Aside from the temperature effect per se the growth rate will be retarded as it pulls away from the first optimal temperature and accelerated as it approaches the second.

This principle is illustrated in Table IV by data obtained by Sherwood in this laboratory. The growth rates were determined at each temperature in medium composed of the following materials:—per 100 cc.: 0.100 K_2HPO_4, 0.100 $CaCl_2$, 0.040 $CaCl_2$, 10 sugar and optimum (1) concentration of ammonium chloride. As stated above it was not necessary to adjust other constituents than the MH_4Cl for temperature changes.

During a certain stage of the growth of yeast in a medium, the rate may be expressed by $\frac{dc}{dt} = kC$ or $\log \frac{C_2}{C_1} = k(t_2 - t_1)$. The data in Table IV are for $\frac{kt_1 + 10}{kt_1} = Q_{10}$. This is compared with data obtained by Slator in a medium composed of sugar and salts with asparagin as the source of nitrogen. It is at once obvious that the figures in the

TABLE III

TEMPERATURE COEFFICIENTS FOR THE GROWTH OF YEAST

Temperature	Medium E optimum	Slator
10	3.5	5.6
15	(2.8)	3.8
20	2.2	2.8
25	(1.8)	2.2
30	1.5	1.9
35	(1.3)	1.6
40	1.0	(1.3)
Variation	2.5	4.3

TABLE IV

AVERAGE TEMPERATURE COEFFICIENTS OBTAINED BY VARIOUS WORKERS

Investigator	Temperature range	Av. Temp. Coeff.
Aberson[1]	12–33	2.72
Herzog[2]	14–28	2.88
Slator[3]	10–35	2.97
Fulmer, et al.	10–40	2.05

[1] Aberson, *Rec. trav. chim.*, 22, 78 (1903).
[2] Herzog, *Zeit. phys. chem.*, 37, 149 (1902).
[3] Slator, A., *J. Chem. Soc.*, 89, 128 (1903).

first column show much less variation than those of Slator. In Table V are given the average temperature coefficients obtained by various investigators of the growth of yeast. It is evident then that the temperature coefficient of reaction in heterogeneous systems varies with concentration of materials in the solution and the effect of temperature alone cannot be determined without appropriately varying concentrations.

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PHYSICO-CHEMICAL STUDIES ON PROTEINS. I.

THE PROLAMINES—THEIR CHEMICAL COMPOSITION IN RELATION TO ACID AND ALKALI BINDING*

by

WALTER F. HOFFMAN AND ROSS AIKEN GORTNER

I. INTRODUCTION

If we except the work of Kossel and his students on the protamines and histones, there has been but little systematic work carried out by a single individual on a single group of proteins. It is true that there are many isolated studies on the preparation and the chemistry of proteins and, of late years, on their physico-chemical properties. In many instances, however, these studies are from different laboratories, and different workers have used somewhat different methods of preparation and analysis. The literature has accordingly become filled with divergent opinions and even statements meant to be statements of fact which it is hard to reconcile with each other. Supposedly duplicate analyses of the same protein by two workers often are widely divergent, perhaps because of different methods used in preparing the protein or in analytical procedure.

Such a condition makes comparisons of even the same protein difficult and comparisons between proteins of different sources all but impossible. Even if we accept all of the available data we are faced with the fact that only a few of the most common proteins have been analyzed for even their nitrogen distribution. No one has attempted to prepare all of the common proteins of a single group, using identical methods so that direct comparison of the properties studied could be made.

This lack of systematic work not only holds true for the chemical analysis of proteins but also for physico-chemical work. At the most,

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a single investigator has carried out identical work on not more than a few different proteins and then only on proteins of altogether different types. Here, again, comparisons between results of different workers is difficult due to differences either in material or manipulative technic.

With these facts in mind, it was our desire to work on one type of proteins, preparing as many proteins as possible in an exactly comparable manner. When such proteins are studied from the chemical and physico-chemical standpoint using identical technic for each protein it should be possible to ascertain whether or not our methods of analysis are sufficiently accurate to differentiate the various proteins within a single group and to demonstrate whether or not the divergent views in the literature are due to actual differences in the proteins or to unavoidable errors of technic. Methods of protein research, imperfect as they still are, have improved very greatly within the past few years. Much of the older work must therefore be carefully repeated in the light of our new knowledge. This paper accordingly represents an attempt to bring together the literature on the alcohol soluble proteins of the cereals, the prolamines, and to study this group of proteins in a comparative manner.

II. HISTORICAL

Part I. The Preparation and Analysis of the Proteins Studied

The Prolamines. The alcohol soluble proteins were among the first proteins recognized in seeds. They have been found in the seeds of all of the cereals examined except rice, the seeds of which were found by Rosenheim and Kajiura (1908) and by Osborne, Van Slyke, Leavenworth and Vinograd (1915) to contain no alcohol soluble protein. None of the alcohol soluble proteins have been found in the seeds of any other family of plants. This group of alcohol soluble proteins, which is one of the best characterized groups in either plants or animals, has been named *prolamines*. This name was proposed by Osborne (1908) in place of "gliadin" which had been suggested earlier. According to the American classification of proteins, the prolamines belong to the division of simple proteins.

The prolamines are characterized by their solubility in dilute alcohol. All are soluble in from 50 to 90 per cent alcohol except zein which is readily soluble in 92 to 93 per cent alcohol. The purified prolamines

are practically insoluble in water but dissolve readily in dilute acid or alkali. They differ from other groups of proteins in that on hydrolysis with acid they yield much glutamic acid, proline and ammonia and little or no lysine.

The principal prolamines thus far prepared are:
Gliadin found in the seeds of wheat, *Triticum vulgare.*
"Gliadin" found in the seeds of rye, *Secale cereale.*
"Gliadin" found in the seeds of oats, *Avena sativa.*
Hordein found in the seeds of barley, *Hordeum vulgare.*
Zein found in the seeds of maize, *Zea mays.*
Kafirin found in the seeds of kafir, *Andropogon sorghum.*

Much work has been done on the preparation and analysis of these proteins. The first serious and systematic work on the preparation and analyses of vegetable proteins was done by Ritthausen. This was followed by the excellent work of Osborne on the preparation of purified vegetable proteins and their analysis. The voluminous literature on the subject prohibits a comprehensive review of the methods of preparation and analyses. Only a few of the more important papers are referred to in the following "historical."

Gliadin from Wheat, *Triticum vulgare.* Although some earlier work had been done on the proteins of wheat, Einhof (1805) is credited with the discovery that a part of the gluten of wheat is soluble in alcohol. He thought that all of the gluten was soluble in alcohol and considered this a characteristic of vegetable proteins. In 1819, Taddei separated wheat gluten into two distinct parts, the alcohol soluble portion he named "gliadin" and the other which was insoluble in alcohol "zymon." The isolation of this protein by extracting wheat meal with hot alcohol was also described by Mulder (1844).

Gliadin is prepared (Osborne, 1907) by extracting either wheat flour or "crude wheat gluten" with approximately 70 per cent alcohol, concentrating the alcoholic solution and pouring it into water containing a little sodium chloride. The precipitated protein, after redissolving in alcohol and again precipitating by pouring this solution into strong alcohol containing a little ether, is dried at a low temperature. It gives all of the usual color tests for proteins.

The average of the elementary analysis made on many samples of gliadin prepared in Osborne's laboratory (Osborne and Harris, 1906) is: carbon = 52.72%, hydrogen = 6.86%, nitrogen = 17.66%, sulfur = 1.14% and oxygen = 21.62%.

The percentages of the various amino acids isolated by different workers from gliadin show great variation:

	Osborne and Clapp (1906)	Osborne and Guest (1911a)	Abderhalden and Samuely (1905)
	Per cent	Per cent	Per cent
Alanine	2.00	1.95	2.66
Amino valerianic acid	0.21		0.33
Glycocoll	0.00	0.00	0.68
Leucine	5.62	6.62	6.00
Valine		3.34	
Proline	7.06	13.22	2.40
Phenylalanine	2.35	1.80	2.60
Aspartic acid	0.58	0.14	1.24
Glutamic acid	37.33	43.66	27.60
Serine	0.13		0.12
Tyrosine	1.20		2.37
Cystine	0.45		
Lysine	0.00		
Histidine	0.61		1.70
Arginine	3.16		3.40
Ammonia	5.11	5.22	
Tryptophane	present		1.00

The nitrogen distribution into seven groups as determined by the Van Slyke analysis is recorded by various workers as follows:

	Van Slyke (1911a)	Osborne, et al. (1915)	Blish (1916)	
			Strong flour	Weak flour
	Per cent	Per cent	Per cent	Per cent
Amide nitrogen	25.52	24.61	26.13	25.90
Humin nitrogen	0.86	0.58	0.50	0.57
Cystine nitrogen	1.25	0.80	0.37	0.29
Arginine nitrogen	5.71	5.45	4.55	4.47
Histidine nitrogen	5.20	3.39	6.77	5.62
Lysine nitrogen	0.75	1.33	0.65	0.97
Mono-amino acid nitrogen	51.98	51.95	53.63	54.10
Non-amino nitrogen	8.50	10.70	7.44	7.55
Total	99.77	98.81	99.97	99.47

There is conclusive evidence that the gliadin prepared from different varieties of wheat, including wheats which give strong and weak flours, are identical. The different varieties of wheat either synthesize identical gliadins or they are modified in some manner during preparation. This identity apparently holds for physical as well as chemical properties.

Wood (1907) was probably the first to suggest that the gliadins from weak and strong flours are identical. He found that the two gliadins have the same amide nitrogen content. These conclusions were substantiated by Blish (1916) when he subjected the individual gliadins prepared from strong and weak flour to the Van Slyke analysis for nitrogen distribution. (The results of his experiments are given in

the above table.) As far as this method shows, the gliadins from strong and weak flours are identical. Cross and Swain (1924), without knowledge of the former work, have recently carried out similar experiments and find that the same results are obtained when gliadins from different flours are subjected to the Van Slyke analysis for nitrogen distribution.

Besides this chemical proof of the similarity of gliadin from different wheats, the physico-chemical determinations yield the same results. Gröh and Friedl (1914) found the gliadin prepared from strong and weak flours to be identical in physical properties. More recently, Woodman (1922) in an extensive study of the physical properties of gliadins from strong and weak flours has come to the same conclusions. He bases his assumption on:

"The identity of their optical behavior when racemised by dilute alkali at 37° C. under three different sets of conditions.

The identity of their specific rotation in 70 per cent alcohol.

The identity of their combining capacities for alkali, as determined by titration in 80 per cent alcoholic solution by means of N/10 sodium hydroxide to phenolphthalein."

The Alcohol Soluble Protein from Spelt, ***Triticum spelta.*** The preparation of "Pflanzenleim" (gliadin), from spelt was described by Ritthausen (1872). The finely ground meal was extracted with dilute alcohol, this solution concentrated, and the protein purified. He gave the following analysis: carbon = 53.69%, hydrogen = 6.83%, nitrogen = 17.71%, and oxygen and sulfur = 21.77%. This protein was also mentioned by von Bibra (1861a). The only analysis given is for sulfur 0.84% as compared with 0.88% in the gliadin of wheat. Apparently no other analysis has been made.

Einkorn, ***Triticum monococcum;*** **emmer,** ***T. dicoccum;*** **and durum,** ***T. durum.*** The alcohol soluble protein has not been previously prepared from these wheat species. These, including *T. vulgare* and *T. spelta* from which the alcohol soluble proteins have been prepared show various characteristics on which the classification of the wheat species is based.

In making a study of sterility in a large number of crosses between these species, Tschermak (1914) places them in three groups as follows:

	Stem species	Cultivated covered	Cultivated naked
Einkorn group	Triticum ægilopoides	T. monococcum	Unknown
Emmer group	T. dicoccoides	T. dicoccum	T. durum T. turgidum T. polonicum
Spelt group	T. spelta wild form unknown	T. spelta	T. vulgare T. compactum

The results of this work with those of Hayes, Parker and Kurtzweil (1920) and of Sax (1921) may be summarized as follows:

1. Einkorn group: *T. monococcum.* All varieties cross fertile; sterile or only slightly fertile with emmer and spelt group.

2. Emmer group: *T. dicoccum, T. durum, T. turgidum* and *T. polonicum.* All species and varieties cross fertile; sterile or slightly fertile with einkorn group; partially sterile with spelt group.

3. Spelt group: *T. spelta, T. vulgare* and *T. compactum.* All species and varieties cross fertile; sterile with einkorn group; partially steril with emmer group.

Again, when classified according to susceptibility to *Puccinia triticina,* the groups as given by Vavilov (1914) are similar to those obtained as a result of sterility studies. They are as follows:

Immune	Resistant	Susceptible
T. monococcum	*T. durum*	*T. vulgare* (few immune)
	T. polonicum	*T. compactum*
	T. turgidum	*T. spelta*

T. dicoccum produced both resistant and susceptible varieties.

That there are three groups of wheat species was also determined by Zade (1914) as a result of serological relationships. These results were obtained by using a serum from animals immunized with the different wheats and determining the reaction with the antigen of the wheats of the same group and those of the different groups.

The wheat groups were also found by Sax (1921) to be separated in a similar way on the basis of pollen size.

Sakamura (1918) and Kihara (1919) reported that these groups likewise differed in chromosome numbers. Sax (1921a) states that he has verified the numbers reported by Sakamura and by Kihara. The haploid numbers are:

Einkorn group	7 chromosomes
Emmer group	14 chromosomes
Spelt group	21 chromosomes

Gliadin from Rye, *Secale cereale.* When Einhof (1805) made his study of the nitrogenous compounds of wheat, he also undertook an analysis of rye, from which he was able to extract an alcohol soluble protein. It was almost 40 years later that Heldt (1843) published a description of the alcohol soluble protein of rye flour. His preparation was obviously impure as is shown by the following analysis. Carbon = 56.38%, hydrogen = 7.57%, nitrogen = 15.83%, and oxygen and sulfur = 19.92%.

In 1866, Ritthausen described three proteins of rye: albumin, "mucedin" (alcohol soluble) and "gluten casein." He considered the "mucedin" as the only protein in rye soluble in alcohol and believed it to be similar to the mucedin he thought was present in wheat. Further

evidence of their identity was furnished by Osborne (1895a) when he prepared pure gliadin from rye using the same method as for gliadin of wheat. He stated that in all of their properties, wheat and rye gliadin resemble each other so exactly as to leave no doubt of their chemical identity. This is shown by the analyses:

	Gliadin (rye) Osborne (1895a)	Gliadin (wheat) Osborne and Harris (1906)
	Per cent	Per cent
Carbon	52.75	52.72
Hydrogen	6.84	6.86
Nitrogen	17.72	17.66
Sulfur	1.21	1.14
Oxygen	21.48	21.62

As stated by Osborne all of the chemical evidence shows the gliadin from wheat and rye to be identical. Besides this, Wells and Osborne (1911) present additional evidence of their similarity. In a study of antiphylaxis induced by vegetable proteins, they found that sensitizing with preparations from one seed yielded as severe symptoms on subsequently intoxicating with those from the other seed as when the same preparation was used for each injection.

In a study of the gliadins from wheat and rye, Lüers (1919) found that the colloid-chemical properties of gliadin from both cereals are identical.

Contradictory evidence has been presented by Gröh and Friedl (1914), who were unable to isolate a preparation from rye identical with that from wheat. Concerning this they state:

"Weizenkleber enthält nur ein einzigens in Alkohol lösliches Protein: Gliadin.

Das aus Roggenmehl extrahierbare Protein ist ein Gemisch mehrerer Eiweissstoffe, deren Isolierung ausserordentliche Schwierigkeiten bereitet.

Aus dem Rnggenmehl gelang es uns nicht, ein mit dem Weizengliadin identisches Präparet zu erhalten.

Es ist unwahrscheinlich, dass im Roggen ein mit dem Weizengliadin identischer Proteinkörper überhaupt vorhanden ist."

Osborne and Clapp (1908) have analyzed rye gliadin by the Fischer method and report the following:

	Per cent
Glycocoll	0.13
Alanine	1.33
Valine	—
Leucine	6.30
Proline	9.82
Phenylalanine	2.70
Aspartic acid	0.25
Glutamic acid	33.81

	Per cent
Serine	*0.06*
Tyrosine	*1.19*
Arginine	2.22
Lysine	0.00
Histidine	0.39
Ammonia	5.11
Tryptophane	present
Cystine	—
Total	64.31

The distribution of nitrogen into the Hausmann numbers as determined by Osborne and Harris (1903) is:

	Per cent of total nitrogen
Ammonia nitrogen	23.42
Humin nitrogen	0.62
Basic nitrogen	4.91
Non-basic nitrogen	71.05

Gliadin from Oats, *Avena sativa.* Probably less is known concerning the alcohol soluble protein of oats than of any of the common prolamines. Norton (1847 and 1848) made a study of the composition of the oat kernel but did not make a special study of this protein. It is impossible to prepare a coherent gluten when oat flour is kneaded with water. In his work on oats, v. Vibra (1861 b) recognized the following alcohol soluble proteins, "casein" 0.15-0.17 per cent, the body separating from hot alcohol on cooling, and plant gelatin (Dumas' "gluten"; Taddei's "gliadin") 3.00-3.25 per cent—the substance soluble in both hot and cold alcohol. This had a nitrogen content of 15.6 per cent.

The preparation and properties of gliadin from oats have been studied by Kreusler (1869) and by Osborne (1891 and 1892). They found that it is very hard to prepare uniform samples of this protein. It apparently undergoes some change when in contact with water or weak salt solutions as this so-called "secondary" protein shows a different composition. Osborne suggests that this alteration of the primary protein is probably due to enzyme action.

Elementary analysis of this protein shows:

	Osborne (1892)		Ritthausen (1872a)
	Extracted directly with alcohol	Extracted with water and then with alcohol	
	Per cent	Per cent	Per cent
Carbon	53.01	53.70	52.29
Hydrogen	6.91	7.00	7.65
Nitrogen	16.43	15.71	17.71
Sulfur	2.26	1.78	1.66
Oxygen	21.39	21.83	20.39

The nitrogen distribution of the prolamine of oats into the various groups as given by the Van Slyke analysis is recorded by Lüers and Siegert (1924) as:

	Gliadin prepared by direct extraction with alcohol	Gliadin prepared extraction first with water and salt solution and then with alcohol
	Per cent	Per cent
Ammonia nitrogen	21.83	18.89
Humin nitrogen	1.19	1.21
Cystine nitrogen	3.77	1.79
Arginine nitrogen	0.53	9.17
Histidine nitrogen	2.15	3.58
Lysine nitrogen	0.15	0.17
Amino nitrogen in filtrate from bases	51.55	51.81
Non-amino nitrogen in filtrate from bases	10.98	11.79

These results agree with the conclusions of Osborne in that the treatment of oats with water and salt solution brings about a change in the composition of the protein. This change is especially noted in the ammonia, cystine and arginine fractions. The percentage of cystine nitrogen in the preparation by direct extraction with alcohol is considerably higher than for any other prolamine thus far analyzed by this method.

Hordein from Barley, *Hordeum vulgare.* The name "hordein" was first used by Proust (1817) for the principles or constituents of barley. It was also used by Hermbstädt (1827) as a name for the products isolated from barley. In 1806, Einhof discovered that a part of the protein of barley is soluble in alcohol. v. Bibra (1861c) named albumen, plant-gelatin and casein (the alcohol soluble protein) as constituents of barley but gave no further particulars concerning these substances other than that they contain on an average 15.5 to 15.6 per cent nitrogen.

Hordein is prepared in the same manner as gliadin from wheat by extracting barley flour with 70 per cent alcohol (Osborne, 1895). It gives all of the usual color tests for proteins except possibly the Molisch test as hordein alone with sulfuric acid gives a pink color which is the same color developed when α-naphthol is present.

The elementary analysis for hordein is:

	Osborne (1895)
	Per cent
Carbon	54.29
Hydrogen	6.80
Nitrogen	17.21
Sulfur	0.83
Oxygen	20.87

The percentages of amino acids isolated from hordein are:

	Osborne and Clapp (1907)	Kleinschmidt (1907)
	Per cent	Per cent
Glycocoll	0.00	0.00
Alanine	0.43	1.34
Valine	0.13	1.40
Leucine	5.67	7.00
Proline	13.73	5.88
Phenylalanine	5.03	5.48
Aspartic acid	—	1.32
Glutamic acid	36.35	41.32
Serine	—	0.10
Cystine	doubtful	—
Tyrosine	1.67	4.00
Oxyproline	doubtful	—
Arginine	2.16	3.14
Histidine	1.28	0.51
Lysine	0.00	0.00
Ammonia	4.87	4.34
Tryptophane	present	present
Total	71.32	75.83

The nitrogen distribution into the Hausmann numbers as determined by Osborne and Harris (1903) is:

	Per cent of total nitrogen
Ammonia nitrogen	23.31
Basic nitrogen	4.47
Non-basic nitrogen	69.96
Humin nitrogen	1.34

Zein from Maize, ***Zea mays.*** The alcohol soluble protein from corn was first prepared by Gorham (1821). He extracted corn meal with alcohol and the clear alcoholic solution was evaporated. A yellow, wax-like substance was obtained. He described it as being soft, ductile, elastic and heavier than water. It was insoluble in water, but soluble in alcohol, turpentine, acids and alkalies. The protein was named "zein," the name by which we now designate the alcohol soluble protein of maize. The yield was 3 per cent. He described zein as resembling gluten but differing from it in containing no nitrogen. He gave no chemical analysis for this protein. It was also reported by Ritthausen (1872b) that maize contained a protein, "maize fibrin" which was soluble in alcohol.

This protein is best prepared (Abderhalden 1910) from finely ground corn or from "corn-gluten," the nitrogen containing by-product of corn starch manufacture. The ground corn or "corn-gluten" is extracted with 70-80 per cent alcohol, the alcoholic solution filtered until clear,

concentrated *in vacuo*, to a small volume and poured into cold water containing a small amount of sodium chloride. The precipitate is then dissolved in 90-93 per cent alcohol. This solution is extracted with ether until fat-free, and then poured into a large volume of water. The precipitate is then dried and powdered. If white corn is used, the product will be white but if yellow corn is used, it is impossible to remove all of the yellow color so that the final preparation will be yellow.

Zein is characterized by its high carbon content, by its resistance to the action of dilute alkalies, and by the ease with which it is converted into an insoluhle modification on being warmed with water or with weak alcohol. Both the soluble and insoluble forms have the same chemical composition and are possibly due to a protein to protean transformation. It gives all of the usual color reactions except the Molisch test and the color reactions for tryptophane.

The elementary analysis for zein is:

	Chittenden and Osborne (1892)	Ritthausen (1872b)
	Per cent	Per cent
Carbon	55.23	54.76
Hydrogen	7.26	7.57
Nitrogen	16.13	15.45
Sulfur	0.60	0.69
Oxygen	20.78	21.47

The amino acid content of zein as reported by various workers is:

	Osborne and Little (1910)	Osborne and Jones (1910)	Dakin (1923)	Kossel and Kutscher (1900)	Langstein (1903)
	Per cent	Per cent	Per cent	Per cent	Per cent
Glycocoll	0.00	0.00	0.00		0.00
Alanine	9.79	9.02 (Alanine and Valine)	3.8		0.50
Valine	1.88				—
Leucine	19.55	18.30	25.0		11.25
Proline	9.04	9.04	8.9		1.49
Phenylalanine	6.55	6.22	7.6		6.96
Aspartic acid	1.71	1.71	1.8		1.04
Glutamic acid	26.17	26.17	31.3		11.78
β-hydroxy glutamic acid			2.5		
Serine	1.02	1.02			
Oxy-proline	—	trace			
Cystine	—	—			
Tyrosine	3.55	3.19	5.2		
Arginine	1.55	1.55		1.82	
Histidine	0.82	0.82		0.81	
Lysine	0.00	0.00		0.00	
Tryptophane	0.00	0.00			
Ammonia	3.44	3.64		2.56	
Total	85.27	80.38			

The nitrogen distribution into the Hausmann numbers is:

	Osborne and Harris (1903)	Gortner and Blish (1915)[1]
	Per cent of total nitrogen	Per cent of total nitrogen
Ammonia nitrogen	18.41	20.75
Humin nitrogen	0.99	0.46
Basic nitrogen	3.04	3.21
Non-basic nitrogen	77.56	77.66

The Alcohol Soluble Protein from Teosinte. The alcohol soluble protein from teosinte seed has never been previously prepared. Teosinte is the Mexican name of a genus of large grasses (*Euchlaena*), the only wild plant which has been hybridized with maize. As pointed out by Collins (1921) it is the nearest wild relative of maize yet discovered and a possible ancestor of that species. As at present recognized, Euchlaena is considered a monotypic genus, all forms of teosinte being referred to the one species, *Euchlaena mexicana* Schrad.

The common teosinte is of the annual type and is grown in southern Florida. It is generally known as "Florida" teosinte. The origin of this type is not known. In regard to the relationship between maize and teosinte, Collins states, "Since the annual forms of teosinte occupy a position between perennial teosinte and maize and since at least one form of annual teosinte is distinguished from the others by characters which it shares with the maize of the region in which it grows, it is suggested that the annual types of teosinte may have originated from hybrids between perennial teosinte and maize."

Kafirin from Kafir, *Andropogon sorghum*. It has been known for some time that kafir contains an alcohol soluble protein. v. Bibra (1861) reports that the seed of Dhurra, a grain sorghum, contains 4.58 per cent alcohol soluble protein but gives no further information concerning it. Osborne (1909) also states that he has found *Andropogon sorghum* to contain such a protein but he apparently did not make a study of it.

This protein, prepared from kafir corn, was named "kafirin" by Johns and Brewster (1916) as it is the first protein isolated from kafir and constitutes the greater portion of the protein contained in the seed. They found that of the 11.7 per cent of protein (N $\times$ 6.25) of kafir, 7.9 per cent could be removed by boiling, 60 per cent alcohol.

In isolating the protein, kafir meal was extracted at about 80° with 70 per cent alcohol, the alcoholic solution filtered, concentrated and poured into a large volume of water. The kafirin first appeared as a milky suspension but the addition of sufficient sodium chloride caused

[1] 48-hour hydrolysis.

the protein to settle out as a bulky, flocculent or granular precipitate, leaving the supernatant liquid almost clear. This precipitate was washed several times by decantation and then filtered off on a cheese cloth. The protein was then extracted with absolute alcohol until free of coloring matter, and then with ether. The product was finally dried at 110°. The yield of pure, dry protein was 5.2 per cent.

The elementary analysis of kafirin as determined by Johns and Brewster (1916) is: carbon = 55.19%, hydrogen = 7.36%, nitrogen = 16.44%, sulfur = 0.60%, and oxygen = 20.41%.

The nitrogen distribution as shown by the Van Slyke method is:

	Per cent of total nitrogen
Humin nitrogen	1.02
Amide nitrogen	20.78
Non-basic nitrogen	71.93
Basic nitrogen	6.25

The percentage of diamino acids in kafirin was also determined by the Van Slyke method. The results have been corrected for the 0.78 per cent of cystine which was precipitated with the phosphotungstates of the other bases.

	Per cent of amino acid in kafirin
Arginine	1.58
Lysine	0.90
Histidine	1.00
Tryptophane	present

Kafirin resembles zein in its ultimate composition but differs in that it is less soluble in cold alcohol and it forms a colloidal suspension when the alcoholic solution is poured into water while zein readily separates. There is a marked difference in the amino acid content of the two proteins as is shown by the nitrogen distribution. The analyses show that kafirin contains lysine and tryptophane both of which are lacking in zein.

The Alcohol Soluble Protein of Sorghum Seed, *Sorghum Vulgare.* v. Bibra (1861) and Osborne (1909) both state that *Andropogon sorghum* contains an alcohol soluble protein but no analyses or other data are given. The only other reference in the literature that has come to our attention where the alcohol soluble protein of sorghum has been mentioned is an article by Visco (1921), who describes its isolation and gives certain properties but gives no analyses except a nitrogen determination.

He prepared the protein from finely ground seed of *Sorghum vulgare.* This meal was extracted several times with 70 per cent alcohol at

55-60° C, the alcoholic solution was filtered, concentrated and poured into several volumes of water containing a little sodium chloride. The protein separated as a gel which slowly settled to the bottom of the container. The water was decanted and the precipitate washed several times with distilled water. Seventy per cent alcohol was then added to the protein but only a part of it went into solution. These portions were then dried and powdered. The one which was soluble in alcohol he designated as A, the other, insoluble in alcohol as B.

Their properties are described as:

A	B
Soluble in alcohol after precipitating in salt solution.	Insoluble in alcohol after precipitating in salt solution.
Insoluble in ether, petroleum ether, chloroform, carbon disulfide and acetone.	Same as A.
Dried at 110° C., insoluble in alcohol.	Same as A.
Heated with concentrated nitric acid, gives a yellow color which becomes more intense when ammonia is added.	Same as A.
Gives sulfur test.	Same as A.
After slight hydrolysis gives biuret test.	Same as A.
Nitrogen = 11.19 per cent.	Nitrogen = 13.61 per cent.

He was able to isolate about 3.5 per cent of protein. The analysis given shows it to contain much less nitrogen than any of the other known prolamines. It is possible, however, that the product was not pure. He does not mention extraction with absolute alcohol or with ether to remove pigments and oils as is necessary in the case of other prolamines in order to obtain a pure product.

It is especially noted that this alcohol soluble protein from sorghum does not give either the Liebermann or Adamkiewicz reaction, showing that it does not contain tryptophane. In this respect it is similar to zein from maize and unlike the kafirin of kafir.

Casein from Cow's Milk. Although casein is not a prolamine, it is used in this work for a comparison with the prolamines because there has been more physico-chemical work done with casein than with any other protein. Casein belongs to the division of phosphoproteins and reacts as an acid whereas the prolamines are either neutral or slightly basic.

The general method for the preparation of casein is to add dilute acid to fat-free milk until an acidity of pH 4.6 is reached. The casein then precipitates and can be separated from the whey by filtering through cheese cloth. It is then washed several times with water at a pH 4.8. Further purification may be affected by dissolving and again precipitating the casein. There are several methods of accomplishing this (Van Slyke and Bosworth, 1913; Van Slyke and Baker, 1918; Hammarsten, 1883, and Cohn, 1922). Pure casein gives all of the protein color tests with the exception of the Molisch reaction.

Casein prepared from cow's milk gives the following elementary analysis:

	Van Slyke and Bosworth (1913)	Hammarsten (1883)	Lehman and Hempel (1894)
	Per cent	Per cent	Per cent
Carbon	53.50	52.96	54.00
Hydrogen	7.13	7.05	7.04
Nitrogen	15.80	15.65	15.60
Sulfur	0.72	0.72	0.77
Phosphorus	0.71	0.85	0.85

After hydrolysis with acid, casein yields the following amino acids:

	Abderhalden (1905)	Osborne and Guest (1911)	Hart (1901)	Foreman (1919)
	Per cent	Per cent	Per cent	Per cent
Glycine	0.0	0.0		0.5
Alanine	0.9	1.5		1.9
Valine	1.0	7.2		7.9
Leucine	10.5	9.4		9.7
Isoleucine	—	—		—
Phenylalanine	3.2	2.8		3.9
Tyrosine	4.5	3.5		—
Serine				0.4
Cystine				—
Proline	3.1	4.7		7.6
Oxyproline				+
Aspartic acid	1.2	1.4		1.8
Glutamic acid	10.7	15.6		21.8
Tryptophane	1.5			
Arginine			4.7	
Lysine			3.2	
Histidine			2.6	
Ammonia		1.6	1.6	

The distribution of the nitrogen in casein as determined by the Van Slyke analysis has been shown to be as follows:

	Van Slyke (1910)	Van Slyke (1914)	Crowther and Raistrick (1916)	Dunn and Lewis (1921)
	Per cent	Per cent	Per cent	Per cent
Ammonia nitrogen	10.43	10.27	10.25	10.49
Humin nitrogen	3.43	1.28	1.20	2.13
Cystine nitrogen	1.95	0.20	1.24	0.48
Arginine nitrogen	7.51	7.41	9.22	7.42
Histidine nitrogen	4.24	6.21	6.82	6.01
Lysine nitrogen	7.86	10.30	9.62	9.09
Amino nitrogen in filtrate from bases	55.04	55.81	54.76	58.78
Non-amino nitrogen in filtrate from bases	9.51	7.13	7.09	5.93
Total	99.97	98.61	100.19	100.33

Fibrin from Blood. Fibrin, a globulin, was also used for comparison with the prolamines. In certain properties it is intermediate between casein and the prolamines. Much work has been done on the chemical and physico-chemical properties of fibrin. Consequently it serves as a check on a part of the work of this paper.

Fibrin is prepared from blood by whipping fresh blood to coagulate the fibrin and then washing the crude strings of fibrin until free of serum and blood cells (Bosworth, 1915). This crude product is purified by solution in dilute alkali, the alkaline solution filtered and the fibrin precipitated by the addition of an exact equivalent of acid. The precipitate is then washed with water containing a trace of sodium chloride. It forms a tough, elastic mass which is very bulky. Salt solutions, alcohol and formaldehyde denature fibrin and destroy its elasticity. The usual protein color tests are given by this protein. The coagulating temperature is 75° C.

Elementary analysis, as reported by Hammarsten (1880) show fibrin to contain, carbon = 52.68%, hydrogen = 6.83%, nitrogen = 16.91%, sulfur = 1.10%, and oxygen = 22.48%.

When fibrin was hydrolyzed with acid, Abderhalden and Voitinovici (1907) isolated the following amino acids:

	Per cent
Glycocoll	3.0
Alanine	3.6
Valine	1.0
Leucine	15.0
Proline	3.6
Phenylalanine	2.5
Aspartic acid	2.0
Glutamic acid	10.4
Serine	0.8
Tyrosine	3.5

A large number of nitrogen distribution determinations by the Van Slyke method have been made on fibrin. The results of four different

determinations are given to show the differences obtained on the same protein by different workers.

	Van Slyke (1911a)	Gortner (1916)	Gortner and Holm (1917)	Gortner and Norris (1923)
	Per cent	Per cent	Per cent	Per cent
Ammonia nitrogen	8.32	10.15	10.14	7.40
Insoluble humin nitrogen	3.17	2.83	2.09	2.07
Soluble humin nitrogen			1.36	0.99
Phosphotungstic acid humin nitrogen			0.31	0.85
Cystine nitrogen	0.99	0.51	27.52 [2]	0.49
Arginine nitrogen	13.86	10.91	10.90	12.72
Histidine nitrogen	4.83	4.36		3.77
Lysine nitrogen	11.51	12.05		12.25
Amino nitrogen in filtrate from bases	54.30	55.43	54.29	54.36
Non-amino nitrogen in filtrate from bases	2.70	2.51	2.50	4.62
Total	99.58	98.75	99.33	99.53

Part II. The Binding of Acid and Alkali by Proteins

Methods of Measuring the Binding of Acid and Alkali by Protein. It has been known for a long time that proteins combine in some form or another with acids and alkalies.[3] The fact that one and the same protein can combine with both acids and bases seems to have been first clearly stated by Platner in 1866. The term "amphoteric" is now used to designate substances possessing this property.

However, the quantitative determination of the extent of acid or alkali binding was dependent upon the development of physico-chemical methods. The results of titrimetric methods to determine the ability of proteins to combine with acids and alkalies was only made clear by the better understanding of the function of indicators. It is only by means of direct determination of the ions present and without disturbing the equilibrium between proteins and the acid or alkali that a clear insight may be obtained as to this combination and of the physico-chemical properties of the products which are present. A few of the attempts at measuring or demonstrating indirectly that proteins combine with acids and bases to form salts are of historical interest as they are the foundation of the more direct physico-chemical methods.

The procedure usually employed for the direct method of demonstrating the existence of protein-acid or base compounds by precipitation or coagulation consists in precipitating, or coagulating, the protein-salt by the addition of suitable reagents. The reagent which is most com-

[2] Total basic nitrogen.
[3] The literature is too voluminous to include here. See Robertson (1906) for a review of the earlier work.

monly used for this purpose is alcohol. The question, however, arises as to whether alcohol precipitates an unaltered protein salt. By using this method to precipitate calcium caseinate, Van Slyke and Hart (1905) found that the precipitated calcium salt was apparently unaltered. In considering the electrochemical phenomena which accompany the coagulation of the caseinates by alcohol, Robertson (1911) points out that coagulation of these salts through the addition of alcohol to their aqueous solutions is preceded by a decrease in their degree of dissociation.

The coagulation of protein salts by reagents other than alcohol is even less reliable. Spiro and Pemsel (1898) employed ammonium sulfate to precipitate sodium caseinate salts. They found that as a maximum, 1 gram of casein precipitated 82×10^{-5} gram equivalents of sodium hydroxide while Robertson (1910), using the potentiometric method which is a static measurement not involving any change in the dynamical condition or composition of the body of the solution, found that in all of the solutions investigated by Spiro and Pemsel, the amount of alkali which was actually bound by 1 gram of casein *while in solution* must have been at least 160×10^{-5} gram equivalents. This is sufficient to indicate the effect of ammonium sulfate on these casein salts.

Direct methods of demonstrating the existence of protein salts or compounds by the solution of otherwise insoluble substances have been employed. W. A. Osborne (1901) has shown that casein, when triturated with calcium carbonate, carries a definite proportion of the calcium into solution in the form of the soluble calcium caseinate with the evolution of carbon dioxide. Similarly, Osborne and Leavenworth (1916) have shown that edestin will hold in solution a quantity of cupric hydroxide corresponding to no less than 35.67 per cent of its weight of copper. Assuming that each atom of copper is united to one nitrogen atom then 10 out of 16 nitrogen atoms are united to a copper atom. This corresponds to the proportion of nitrogen atoms of edestin which are supposed to be present in the form of *—CONH—* groups. Identical results were obtained with gliadin.

A very ingenious method of determining the acid binding capacity of proteins, based upon the fact that phosphotungstic acid forms insoluble salts with proteins, has been worked out by Cohnheim and Krieger (1900). If the protein is combined with an acid and calcium phosphotungstate is used, double decomposition takes place according to the following scheme:

$$\underset{\text{(soluble)}}{\text{Protein hydrochloride}} + \underset{\text{(soluble)}}{\text{calcium phosphotungstate}} =$$

$$\underset{\text{(insoluble)}}{\text{protein phosphotungstate}} + \text{calcium chloride.}$$

The quantity of calcium chloride present in the filtrate is taken as a

measure of the quantity of hydrochloric acid which was bound by the protein just before precipitation.

The indicator method of determining the amount of acid or alkali bound by a protein has also been extensively used but due to the complexities which arise, the results have, in many cases, been erroneously interpreted. The final result obtained with a given indicator tells nothing save the condition of equilibrium in the solution at the precise hydrogen ion or hydroxy ion concentration at which that indicator changes color. Neither does it give indication as to the acid or alkali binding capacity of the protein at any other hydrogen or hydroxyl ion concentration.

The two principal drawbacks accompanying the use of indicators in protein solutions are:

1. Owing to the amphoteric character of the protein and also to their multiple combining capacity, the change of hydrogen or hydroxyl ion concentration when acid or alkali is added is not very great, thus the end points are not sharp.

2. Many of the substances used as indicators are either acids or bases. They may combine chemically with the proteins.

The first attempts to use physico-chemical methods in determining the amount of acid or alkali bound by a protein were made by measuring the electrical conductivity of the protein solution to which varying amounts of acid or alkali had been added. Among the first to use this method were Sjöqvist (1895 and 1895a) and Laqueur and Sackur (1903). The latter authors were the first to use modern physico-chemical methods in studying the effects of adding alkali to casein. Although this method has been employed by many investigators, it gives but a limited insight into the behavior of proteins with acids or alkalies. As long as the rapidly moving hydrogen ion is taken out of solution by the protein, the specific conductivity will fall rapidly. This is a very indirect and uncertain method of getting at the amount of acid or alkali that combines with the protein. A very slight excess of acid or alkali will greatly change the conductivity. Furthermore, the mobility of the charged protein particle is nowise independent of the quantity of acid or alkali combined.

The cryoscopic method has also been used to measure the binding of acids and alkalies by proteins. This measurement is also a static one which differs from the former one in that the measurement is necessarily conducted at or near 0° C. and the equilibrium studied is accordingly shifted to the equilibrium at this temperature.

This method was first employed by Bugarszky and Liebermann (1898). They found that when 6.4 grams of egg albumin was added to 100 cubic centimeters of 0.05 normal hydrochloric acid or sodium hydroxide, the difference between the freezing point of the solution and that of water is reduced approximately 50 per cent, thus indicating

a diminution of nearly 50 per cent in the total number of ions plus molecules. They also point out that when protein is added to a solution of sodium chloride no appreciable alteration is noted in the freezing point. Hardy (1905), however, has detected a slight but seemingly unmistakable decrease in the electrical conductivity from which he assumed that a compound is actually formed with the neutral sodium chloride. Similar conclusions have been put forth by Mellanby (1905).

More recently Takeda (1922) has employed the cryoscopic method for studying the binding of acids and alkalies by albumin. He finds that a 0.75 per cent albumin solution in 0.004 normal hydrochloric acid causes no change in the depression of the freezing point from that of the acid alone. This, according to Takeda, indicates that there are as many free chlorine ions as albumin ions. If the concentration of hydrochloric acid is increased, the depression of the freezing point is less than that of the acid alone. Similar results were obtained when albumin was added to sodium hydroxide. In this case, however, it was found that the depression of the freezing point was increased slightly, indicating that the "sodium albuminate" is more highly dissociated than is sodium hydroxide. When ammonium hydroxide was used, a much greater increase in the depression of the freezing point was noted, showing that the ammonium salt of albumin is much more highly ionized than is ammonium hydroxide.

The important work of Hoffmann (1889 and 1890) and Cohnheim (1896) who measured the concentration of hydrogen ions in solution by the acceleration of the inversion of cane sugar, failed only on account of the slight sensitivity of the method. This same principle was employed by Hardy (1905) who used the catalysis of the inversion of cane sugar by hydrogen ions in the measurement of the acid binding capacity of serum globulin, and the saponification of methyl acetate by hydroxy ions in the measurement of the alkali binding capacity of this protein. Winten and Krüger (1921) also used the rate of saponification of methyl acetate to measure the amount of alkali not bound in a gelatine-sodium hydroxide mixture.

Burgarszky and Liebermann (1898) made the next notable step in measuring the amount of acid or alkali bound by a protein. These investigators introduced the electrometric method for measuring the hydrogen ion and chlorine ion concentration of a solution. A study of their results shows that a water solution of protein binds hydrochloric acid and sodium hydroxide but not sodium chloride. In the case of hydrochloric acid, it was found that chlorine ions as well as hydrogen ions were bound by the protein. They also point out that the rate of binding decreases as the amount of protein added, is increased.

This method is the basis of much of the work which has been done on the acid and alkali binding capacity of proteins. In a careful study of the binding of hydrochloric acid by proteins, Manabe and Matula

(1913) found that the amount of acid bound by a definite amount of protein increases to a maximum and remains at this maximum even though more acid is added. The acid is never completely bound but there is always some residual free acid. They also show that when solutions of serum protein and hydrochloric acid are diluted, the amount of bound acid decreases. Shortly after this, Blasel and Matula (1913) published the formula:

$$n = N - \frac{cH}{\alpha}$$

where n = the amount of acid or alkali bound,
N = the original normality of acid or alkali.
cH = the measured hydrogen ion concentration of the protein-acid or -alkali solution at equilibrium,
α = the dissociation constant as calculated from specific conductivity data,[4]

for calculating the amount of acid or alkali bound by a protein.

In experiments, carried out by Pauli and Spitzer (1922) using potentiometric measurements over a wide range of concentrations of albumin and alkali, the combined portion of the alkali is calculated by using the same formula in the case of strong alkalies, as for acids,

$$n = N - \frac{cOH}{\alpha}$$

However, for weak bases it can be derived by means of the dissociation constant from the expression,

$$n = \frac{K(N - cOH) - (cOH)^2}{K - cH}$$

In an attempt to obtain further information as to both the general and special quantitative relations holding between the gelatine (base) and its combined acid in acid systems, and between the gelatine (acid) and its combined base in alkaline systems respectively, Lloyd and Mays (1922) report some very interesting results. They show that hydrochloric acid combines with gelatine in solutions whose acid concentration is less than 0.04 normal, according to the law of mass action. The theory is advanced that over this range of the curve of combination of gelatine with hydrochloric acid, the combination occurs at the free amino groups. They consider that these groups are present in lysine and arginine and possibly some other amino acids of gelatine. The salts formed are said to be hydrochlorides. When the concentration of hydrochloric acid is greater than 0.04 normal, the proportion of acid

[4] As will be shown below, the value for α as calculated from conductivity data cannot be used in this formula to give a correct value for n.

fixed is greater than would follow from the combination of hydrochloric acid with a weak base. This combination is not due to a hydrolytic decomposition of the gelatine, thus releasing more amino groups. It is suggested that combination also occurs at the nitrogen of the peptide linkages.

Their curve (Lloyd and Mays, 1922, Fig. 4) of acid bound plotted against hydrogen ion concentration is not a simple, smooth curve but consists of two, and possibly three distinct portions. They suggest that the first portion represents the free amino groups of lysine and arginine, the second corresponds to the imino nitrogen of the bases, *i.e.*, arginine and lysine together with histidine, and the third is not distinct but it undoubtedly represents some of the CONH groups combining with acid.

Other workers, however, have found that the combination curve for a protein and an acid does not form this irregular line but that it formed a smooth curve. Loeb and Pauli have both done a large amount of work on the combination of acids with proteins and find that the combination curve is smooth and regular and that it flattens off at the higher concentrations of acid. Hitchcock (1922a) has found that this curve flattens at about pH 2, where 1 gram of gelatine binds about 0.00092 gram equivalents of hydrochloric acid. He calculated the amount of bound acid by titrating water with hydrochloric acid and determining the amount of acid required to obtain a certain pH. The difference between this and the amount of acid required to bring a gelatine solution to the same pH gives the amount of acid bound by the protein. The same method was earlier used by Tague (1920) for determining the amount of alkali neutralized by amino acids.

Calculation of Acid and Alkali Binding by Proteins. In calculating the amount of hydrochloric acid bound by gelatine, Lloyd and Mays used the formula,

$$n' = N - \frac{(H^+)corr.}{\alpha}$$

where n' = the normality of acid bound,
N = the original normality of acid,
$(H^+)corr. = \sqrt{(H^+)(Cl^-)}$
α = the degree of ionization of the acid as determined by conductivity data.

The values which they used for the degree of ionization were from conductivity data given by Lewis (1920). Where the amount of free hydrochloric acid is calculated from these values of α and the hydrogen ion concentration measured by potentiometric methods, the correct normalities, *i.e.*, the amount of acid removed from the solution by the protein, are not obtained.

Vosburgh and Eppley (1923) in a review of "The Determination of Hydrogen Ions," by Clark, state, "Sörensen assumed that conductance data gave the correct degree of dissociation for 0.1 M hydrochloric acid solution and based his pH values on that. Recent work on activities has shown that Sörensen's assumption was undoubtedly in error. . . ." Our experimental data (see "Experimental") show these postulations to be correct. If the values for α as used by Lloyd and Mays are compared with those obtained from potentiometric measurements a marked difference is noted. This difference is much greater in the case of sodium hydroxide than in the case of hydrochloric acid. *The degree of ionization of acid and alkali as determined by conductivity measurements and by potentiometric methods do not agree. The values from conductivity data cannot be used for calculating the amount of acid or alkali bound by a protein.* The formula, therefore, must become either,

$$n = N - \frac{cH}{\alpha'} \text{ or } n = N - \frac{cH corr.}{\alpha'}$$

where α' is the degree of ionization determined potentiometrically.

In connection with Hitchcock's method of calculating the amount of acid bound by a protein, the assumption is made that the same concentration of uncombined hydrochloric acid is required to give the same *pH* to equal volumes of water and of protein chloride solutions. The same assumption was made by Blasel and Matula (1913) when they used the formula,

$$n = N - \frac{(H^+)}{\alpha}$$

but Lloyd and Mays considered that a closer approximation for the value of H^+ is obtained by taking the square root of the product of the hydrogen ion and chlorine ion concentrations as the basis of the calculation. The value for (H^+) is determined experimentally and the value for (Cl^-) is obtained by assuming that the gelatine chloride is completely ionized so that $(Cl^-) = (H^+) + n$. The value for (H^+) in the above formula then becomes, $\sqrt{(H^+)(Cl^-)}$. The true value must lie somewhere between these two, *i.e.*, (H^+) or (H^+) *corr.*

The assumption that none of the chlorine ions are bound by the protein was also made by Procter and Wilson (1916) although the experimental work of Burgarszky and Liebermann (1898) shows that in the case of albumin almost as many chlorine as hydrogen ions are bound. The results of Burgarszky and Liebermann are substantiated by Manabe and Matula (1913), who found that the ionization of the protein chloride increased to a maximum in about 0.02 normal hydrochloric acid and then fell off until at a concentration of about 0.05 normal it is almost completely suppressed. This phenomenon indicates that the free acid which accumulates on further acidification, after

saturation of the protein, finally almost completely suppresses the ionization of the protein chloride. The ionization of protein chlorides, as determined by Hitchcock (1923a) agrees fairly well with the results of the earlier work. He showed that at the lower concentrations of hydrochloric acid almost complete ionization occurred while at the higher concentrations (final pH of about 1.08) there is no appreciable ionization in the case of gelatine chloride, approximately 50 per cent in the case of egg albumin chloride and edestin chloride and almost none in the case of serum globulin chloride.

From a consideration of the above evidence, the assumption that the same concentration of uncombined acid is required to give the same hydrogen ion concentration to equal volumes of water and of protein chloride solutions appears to be more correct than to assume that the protein chloride is completely ionized. The experimental data show that it is only at the lower concentrations of acid where considerable ionization occurs and at these concentrations the correction for the chlorine ion is very small. At the higher concentrations of acid, the correction for the chlorine ion is quite large but the ionization is very small (as a rule less than 50 per cent). The true value for the amount of acid bound appears to be much nearer the value obtained from the formula,

$$n = N - \frac{H^+}{\alpha'} \quad \text{than from} \quad n = N - \frac{H^+_{corr.}}{\alpha'}$$

where α' is the degree of ionization as determined by potentiometric methods.

Types of Combination of Acid and Alkali with Proteins. - There are two ways in which the ions of an acid, for example hydrochloric acid, may combine with proteins. They may either combine stoichiometrically, *i.e.*, by the purely chemical forces of primary valency as postulated by Loeb, or, according to the empirical rule of adsorption, as is assumed in colloid chemistry.

In his work on the binding of acid by proteins, Loeb (1922) states, "these titration experiments then leave no doubt that these acids (hydrochloric, sulfuric, oxalic and phosphoric) combine with proteins in the same stoichiometric way as they combine with crystalloids. That these simple facts had not been discovered earlier is the consequence of the failure of the workers to consider the hydrogen ion concentration of their solutions. Had this been done, nobody would have thought of suggesting that acids combine with proteins according to the adsorption formula." Apparently Loeb considered that the "colloid chemist" denied the chemical combination of acids and alkali with proteins and that they attempted to explain all phenomena by adsorption. There are certainly but very few who take this view. Loeb objected to the introduction of aggregation and adsorption but admitted all other

physical considerations such as membrane effects and electric charges.

As proof of this chemical combination, Loeb showed that the same number of cubic centimeters of 0.1 normal hydrochloric and sulfuric acids and three times as many cubic centimeters of 0.1 normal phosphoric acid are required to bring 1 gram of egg albumin to the same pH. The ratio of hydrochloric acid to oxalic acid is a little less than 1:2, when the hydrogen ion concentration is greater than pH 3. These combining ratios of the four acids with egg albumin are considered to be the same as those which would be found if the crystalloid base ammonia was substituted for the protein and titrated in the same range of pH. Similar data are presented for other proteins and the same relationship is found to hold.

From these data, the amount of acid combined with the protein is calculated. As further proof of the stoichiometric character of the combination of acids with albumin, it is shown (Loeb, 1922) that the same mass of protein combines with three times as many cubic centimeters of 0.1 normal phosphoric acid as hydrochloric and sulfuric and with twice as many cubic centimeters of 0.1 normal oxalic acid, below pH 3. This relationship is the same for other proteins. Loeb also showed that for all strong monobasic acids like hydrobromic and nitric, the titration curve is the same as for hydrochloric acid. This does not hold in the case of the weaker acids as a greater quantity of acid is required to bring the protein solution to the same pH. Due to the enormous quantities of weak acid required, it was not possible for Loeb to plot the quantity of acid in combination with a given mass of protein in the same way as in the case of hydrochloric acid. By an indirect method, he showed that the amount of anion combined with a given amount of protein in the same volume of solution is the same for a given pH, regardless of the strength of the acid.

Similar results were obtained when proteins were titrated with alkali. The same quantity of 0.1 normal alkali is required to give a protein solution the same pH, *i.e.*, sodium, potassium, barium and calcium hydroxides all combine in equivalent proportions with proteins in the same stoichiometric way in which they combine with crystalloid bases.

Hitchcock (1922) obtained similar results with edestin. He found that the same number of cubic centimeters of 0.1 normal hydrochloric and sulfuric acids, twice as much 0.1 normal oxalic acid, and three times as much 0.1 normal phosphoric acid combined with equal amounts of edestin at the same pH. The values for hydrochloric, sulfuric and oxalic acid were obtained by difference between titration curves with and without protein. The values for phosphoric acid were calculated from the first ionization constant, assuming complete ionization of the edestin phosphate. If, however, the values for the amount of phosphoric acid combined are obtained in the same manner as for the other acids, not three but almost five times as many cubic centimeters of phosphoric

as hydrochloric acid are combined. This does not agree with Loeb's results as he found the ratio between hydrochloric, sulfuric and phosphoric acid to be 1 : 1 : 3 when the amount of phosphoric acid was calculated by difference, while, by the same method of calculation Hitchcock's results show a ratio of about 1 : 1 : 5.

This is a brief review of the evidence upon which Loeb has "proven" that proteins are amphoteric electrolytes and react stoichiometrically with acids and bases to form salts capable of electric dissociation. No reference is made to the work on the "adsorption theory" of the binding of acids and alkalies by proteins. Neither is evidence given to show that this "chemical combination" is not according to the empirical rule of adsorption as is held by some workers.

In a discussion of Loeb's work, Bayliss (1923) states, "The work of Loeb has undoubtedly brought to light many important facts under the particular conditions of his experiments, but there are reasons for doubt as to whether all the properties of proteins can be explained by combination with acids and bases in equivalent proportion, apart from changes in physical state or adsorption. The intervention of the Donnan membrane equilibrium when other electrolytes are present is also thoroughly worked out and its importance emphasized. One would like, however, a more detailed investigation of the behavior of uncombined proteins in contact with pure water, where the Donnan membrane equilibrium does not intervene. Also of the osmotic and electrical properties of protein salts in absence of foreign salts or other electrolytes. Here also the Donnan membrane effect would not complicate matters."

Greenberg and Schmidt (1924) have very recently published evidence which supports the view that there is chemical combination between acids and alkalies and proteins. Using Hitchcock's data (1923), they show that the amount of acid bound by gelatin is equal to the sum of the free amino nitrogen plus the amino group of arginine which does not react with nitrous acid. This undoubtedly holds for the particular temperature and concentration of acid used. It will be shown later that both temperature and concentration of acid have a marked effect in influencing the "maximum binding capacity" of proteins.

The seat of alkali binding is assumed to be at the dicarboxylic acids. The calculations are based on the fact that the second carboxyl group of glutamic and aspartic acid is not completely neutralized by ammonia to form $CONH_2$. The discovery of β-hydroxy-glutamic acid by Dakin (1918) and the work of Harris (1923) which indicates that tyrosine must be regarded as a dibasic acid, requires that these acids be considered with glutamic and aspartic acids. The calculated amount of alkali which should be bound by a protein should be the sum of alkali bound by glutamic and aspartic acid, β-hydroxy-glutamic acid and tyrosine minus the amid nitrogen. Greenberg and Schmidt (1924)

have found a direct relationship between the calculated amount and that actually bound.

Their results for the binding of sodium hydroxide are:

Casein,	mols of sodium hydroxide which should be bound........	158×10^{-5}
	mols of sodium hydroxide 1 gram binds at pH 12........	160
Gelatin,	mols of sodium hydroxide which should be bound.........	42
	mols of sodium hydroxide 1 gram binds at pH 11........	60
Gliadin,	mols of sodium hydroxide which should be bound.......	34
	mols of sodium hydroxide 1 gram binds at pH 11........	30

From these results it is seen that a hydroxyl ion concentration of pH 12 is required to give the amount of alkali bound by casein which is equal to that which should be bound as calculated from the four amino acids named above. For gelatin and gliadin a hydroxyl ion concentration of only pH 11 is required to bring about the necessary binding. It would appear that *if their reasoning is correct the same final pH should hold with each protein.*

There has been a large amount of evidence submitted in favor of the adsorption theory or at least that the combination of acids and alkalies with proteins takes place according to the empirical adsorption formula. There are reasons why the true adsorption of ions alone does not give a satisfactory explanation of this phenomenon. These reasons are based chiefly on the osmotic pressure. When an acid or alkali is added to a protein, there is a greater increase in osmotic pressure than is due to the electrolytes. If the reaction is mere adsorption, there would not be the increase in total active elements which the results of osmotic pressure determinations require. The evidence afforded by osmotic pressure is very well summarized by Bayliss (1923), "There is sufficient evidence that the osmotic pressure which proteins show in acid or alkaline media is due chiefly or entirely to the inorganic ion, which can only be effective if kept within the membrane by the electrostatic attraction of the opposite indiffusible ion of an electrolytically dissociated salt, itself indiffusible. Thus, there is no doubt of the existence of such salts. But, it may be remarked incidentally, the protein ion is largely aggregated and must possess the properties of surface. If this be so, it is capable of taking up other substances by adsorption. By this means the concentration of these substances is lowered and important results may follow. Change in the degree of dispersion of proteins would also regulate the concentration of adsorbed substances in the external phase."

Hammersten (1877) was probably one of the first to suggest that this combination is not chemical as he was able to remove all of the acid from a casein-acid solution by repeated washing. According to

the modern conceptions of balanced reactions, it does not necessarily hold that he was not dealing with a chemical combination.

Experimental evidence of the adsorption theory is furnished by Van Slyke and Hart (1905) and Van Slyke and Van Slyke (1907) who have described a series of compounds of casein with various inorganic acids. They find that when 1 gram of casein is suspended in 100 cubic centimeters of N/500 acid at 0° C. it takes up 17.4×10^{-5} equivalents of sulfuric acid, 11.9×10^{-5} equivalents of hydrochloric acid, 8.9×10^{-5} equivalents of lactic acid or 5.3×10^{-5} equivalents of acetic acid. When the temperature is raised, the rate at which equilibrium is approached increases but the final amount of acid taken up decreases. Van Slyke and Van Slyke (1907 and 1908) believe that the binding of acids by casein, under these conditions, is an "adsorption" phenomena.

In studying the binding of chlorine by a protein, Oryng and Pauli (1915) found that this combination follows the empirical adsorption formula. They observed that when the original chlorine ion concentration is plotted against the amount of chlorine ions bound, an adsorption curve is obtained and when the original pCl was plotted against the pCl bound, a straight line was obtained showing that the binding of the chlorine ions follows the adsorption law. Experiments were carried out with serum protein, gelatin, deaminized gelatin and amino acids. All of the curves show that this follows the adsorption formula.

Tolman and Stearn (1918) determined the amount of acid adsorbed by suspending fibrin in a solution of hydrochloric acid for a definite period and then titrating the remaining hydrochloric acid. They found that the hydrogen ions were adsorbed from the solution. It is suggested by these authors that the theory would be equally applicable, if the adsorption process should lead to a fairly uniform coating of ions over the whole of the exposed surface, or, on the other hand, if the hydrogen ions should only tend to go on to the protein at special points, where there is a particularly strong stray field, for example, where the amino and acid groups of the amino acids come together. Similar adsorption curves were obtained by Herzog and Adler (1908) using hide powder and by Procter (1914) using gelatin. Tolman and Bracewell (1919) report the adsorption of alkalies by fibrin to be similar to that of hydrochloric acid and state that it can be explained by the same theory. In a theoretical discussion of this adsorption phenomenon, Bracewell (1919) considers this attraction to be of a chemical nature, arising from stray fields of "chemical force" around atoms whose chemical affinity has not been completely satisfied. Probably the most conclusive evidence in favor of the adsorption theory is the work of Izaquirre (1923). He used the data of Lloyd and Mays (1922) who determined the amount of hydrochloric acid

bound or adsorbed by a definite amount of gelatine when the gelatine was placed in different concentrations of acid. The concentration of acid not adsorbed was measured electrometrically. His calculations show that a logarithmic relationship exists between the amount of acid bound and the concentration, thus resembling the usual adsorption reaction.

It has been shown by Pauli and Hirschfeld (1914), Loeb (1920), Procter (1911) and Hitchcock (1922) that approximately equal gram equivalents of hydrochloric and sulfuric acid combine with unit quantities of protein. The combination of albumin with acetic acid falls considerably short of that which occurs with strong acids. (Pauli and Hirschfeld, 1914.) This, however, is only true when acids of the same normal concentrations are compared. If solutions of the same hydrogen ion concentration are compared, the quantity of acetic acid which combines is considerably greater. For example, a 0.2 normal acetic acid solution corresponds (in hydrogen ion concentration) to a 0.002 normal hydrochloric acid solution but more than four times as much acetic acid is bound at this hydrogen ion concentration. According to their work, the same amount of acetic and hydrochloric acid are not bound by equal amounts of a protein at the same pH as claimed by Loeb.

Mode of Acid and Alkali Combination with Proteins. From the results of the conductivity and potentiometric measurements on proteins plus acid or alkali, it is obvious that some of the acid is bound by the protein. Assuming this combination to be of a chemical nature rather than of a purely physical type, there are two ways in which the combination may take place.

The earlier workers held the view that proteins form typical metallic salts with bases. This conception was adhered to by Hardy and by Pauli and may be expressed in the following equation, in which the free carboxyl groups react as acid valencies with the formation of a negative protein ion,

$$R\left\langle\begin{array}{l}NH_2\\COOH\end{array}\right. + NaOH \leftrightarrows R\left\langle\begin{array}{l}NH_2\\COONa\end{array}\right. \rightleftarrows R\left\langle\begin{array}{l}NH_2\\COO^-\end{array}\right. + Na^+$$

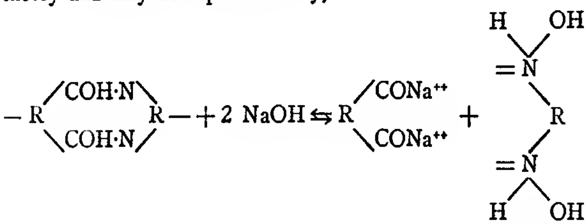
or in which the free amino groups react as alkali valencies with the formation of a positive protein ion,

$$R\left\langle\begin{array}{l}NH_2\\COOH\end{array}\right. + HCl \leftrightarrows R\left\langle\begin{array}{l}NH_2HCl\\COOH\end{array}\right. \leftrightarrows R\left\langle\begin{array}{l}NH_2H^+\\COOH\end{array}\right. + Cl^-$$

Robertson (1918) has also emphasized the importance of the peptide linkage as the source of basic and acid valencies, and bases his theory on the following scheme, in which the peptide group reacts in the enol form,

$$-\mathrm{COH{\cdot}N}- + \mathrm{NaOH} \leftrightarrows -\mathrm{CONa^{++}} + =\overset{\displaystyle \mathrm{H}}{\underset{\displaystyle \mathrm{OH}}{\mathrm{N}}}-$$

According to his theory, the protein is ionized by rupture of the peptide linkage with formation of two oppositely charged protein fragments. The diamino and dicarboxyl groups also play a leading part in this theory and may be represented by,



This is sufficient to show that the salts formed according to the Robertson theory could give no free inorganic ions but only an equal number of oppositely charged protein ions. If these protein salts are subject to electrolysis, the protein salts with alkali show only negative protein ions, in the same way as when acid is added, migration is to the cathode only. The proof that proteins are amphoteric electrolytes which form regular salts with acids and alkalies and that these salts are capable of electrolytic dissociation is very well summed up by Pauli (1922) (using an alkali proteinate as an example) as follows:

"The protein part of the alkali protein migrates to the positive pole, while the alkali content increases at the negative pole.

"The viscosity of solutions of the protein salts passes through a maximum value corresponding to a repression of the ionization of the alkali protein in excess of alkali—a behavior exactly parallel to that of acid albumin in excess of acid.

"The difference in electrical conductivity between sodium caseinate and ammonia and potassium caseinates corresponds to the difference in mobility of the sodium ion compared with the ammonia and potassium ions. This behavior is compatible only with the existence of free metal ions.

"When casein is added to sodium hydroxide solution, a fall in the total molecular concentration of the latter occurs which can be demonstrated by the diminution of the freezing point depression. This effect is produced by the poly acid character of the casein, as a result of which only one polyvalent casein ion appears when several hydroxyl ions of the alkali are replaced."

Further evidence that protein salts behave as amphoteric electrolytes

which are capable of ionization is shown by the difference in the amount of hydrogen and chlorine ions bound by the protein. If the theory put forth by Robertson is correct, the amount of hydrogen and chlorine ions bound by the protein would be equal.

Maximum Binding Capacity of Proteins. The question next arises as to the amount of acid or alkali bound by a protein. It is beyond the scope of this paper to give a review of all the quantitative work that has been done on the binding of acid and alkali by proteins. Some of the values that have been given for the maximum binding capacities and for the binding at different hydrogen ion concentrations are given below. Here, again, comparison is extremely unsatisfactory in some cases, as the methods used are so different.

When the protein is neutralized (using litmus),

One gram of casein neutralizes approximately 55×10^{-5} equivalents of alkali. (Van Slyke and Hart, 1905; Söldner, 1895; and Robertson, 1910.)

One gram of ovomucoid neutralizes 7×10^{-5} equivalents of hydrochloric acid. (Robertson, 1910a.)

One gram of egg albumin neutralizes 14.5×10^{-5} equivalents of alkali. (Osborne, 1899.)

When the protein is neutralized (using phenolphthalein),

One gram of casein neutralizes about 90×10^{-5} equivalents of alkali. (Bosworth and Van Slyke, 1913; Van Slyke and Hart, 1905; Söldner, 1895; Laqueur and Sackur, 1903; Courant, 1891.)

One gram of fibrin neutralizes 61.5×10^{-5} equivalents of alkali. (Bosworth, 1915.)

One gram of egg albumin neutralizes 22×10^{-5} equivalents of alkali. (Osborne, 1899.)

When the protein is neutralized (using tropaeolin) (Osborne 1899a),

One gram of edestin neutralizes 130×10^{-5} equivalents of hydrochloric acid.

One gram of excelsin neutralizes 124×10^{-5} equivalents of hydrochloric acid.

One gram of legumin neutralizes 130×10^{-5} equivalents of hydrochloric acid.

One gram of amandin neutralizes 103×10^{-5} equivalents of hydrochloric acid.

One gram of egg albumin neutralizes 90×10^{-5} equivalents of hydrochloric acid.

When acid or alkali is "saturated" with the protein:

One gram of serum globulin neutralizes 18×10^{-5} equivalents of acid. (Hardy, 1905.)

One gram of casein neutralizes 32×10^{-5} equivalents of hydrochloric acid. (Robertson, 1909.)

One gram of casein neutralizes 11.5×10^{-5} equivalents of alkali. (Robertson, 1909; Van Slyke and Bosworth, 1913a.)

One gram of edestin neutralizes 14.0×10^{-5} equivalents of acid. (Osborne, 1901.)

One gram of edestin neutralizes 7.0×10^{-5} equivalents of alkali. (Osborne, 1901.)

The maximum amount of acid or alkali bound by a protein:

One gram of ovomucoid binds 50×10^{-5} equivalents of potassium hydroxide. (Robertson, 1910a.)

One gram of ovomucoid binds more than 100×10^{-5} equivalents of acid. (Robertson, 1910a.)

One gram of gelatin binds 130×10^{-5} equivalents of acid or alkali. (Procter and Wilson, 1916.)

One gram of gelatin binds 119×10^{-5} equivalents of acid or alkali. (Winten and Krüger, 1921.)

One gram of gelatin binds 92×10^{-5} equivalents of hydrochloric acid. (Hitchcock, 1922a.)

One gram of gelatin binds 300×10^{-5} equivalents of hydrochloric acid. (Lloyd and Mays, 1922.)

One gram of gelatin binds 150×10^{-5} equivalents of hydrochloric acid. (Manabe and Matula, 1913.)

One gram of gelatin binds 152×10^{-5} equivalents of hydrochloric acid. (Blasel and Matula, 1913.)

One gram of deaminized gelatin binds 44×10^{-5} equivalents of hydrochloric acid. (Hitchcock, 1923.)

One gram of deaminized gelatin binds 120×10^{-5} equivalents of hydrochloric acid. (Blasel and Matula, 1913.)

One gram of gelatin binds about 2000×10^{-5} equivalents of sodium hydroxide. (Lloyd and Mays, 1922.)

One gram of deaminized gelatin binds about 210×10^{-5} equivalents of sodium hydroxide. (Blasel and Matula, 1913.)

One gram of casein binds 180×10^{-5} equivalents of alkali. (Robertson, 1910.)

One gram of serum albumin binds 166×10^{-5} equivalents of acid. (Manabe and Matula, 1913.)

One gram of serum albumin binds 167×10^{-5} equivalents of acid. (Pauli and Hirschfeld, 1914.)

It is plainly seen that there is no agreement by the various authors,—a fact which justifies an extensive study of the comparative behavior of a large number of proteins by the same worker and using identical methods.

The Isoelectric Point. Levene and Simms (1923) define the iso-

electric point of an amphoteric substance as that hydrogen ion concentration at which it is ionized equally as an acid and as a base: It may also be considered as the point of minimum dissociation. In consequence, it is the hydrogen ion concentration at which the particles will not migrate in an electrical field. Cohn (1921) has suggested that the isoelectric point is the point on the titration curve where no acid or alkali is bound.

It was pointed out by Michaelis (1914) and by Levene and Simms (1923) that in the case of ampholytes as some of the amino acids and proteins, the isoelectric point is very definite and in these cases it can be readily calculated. If, on the other hand, there is no dissociation over a large range, their formulæ cannot be used. There is in reality an *isoelectric range* in which there is no change in properties either physically or from the standpoint of ionization.

This appears to be the case in most of the proteins, *i.e.*, a very small amount of alkali will greatly increase the pH if the protein was originally near its isoelectric point. There is a wide range in pH over which the proteins act, but slightly, as buffers. Bayliss (1923) has observed that proteins have an isoelectric range rather than a definite isoelectric point. It is very difficult to measure any physical changes over this range.

This isoelectric range is shown by the buffer curves for proteins plus acid and alkali (Lloyd and Mays, 1922 and Loeb, 1922). Eckweiler, Noyes and Falk (1921) have shown that amino acids and peptides behave in a similar manner. Gortner and Sharp (1923) have found that the viscosity of a flour suspension falls to a minimum at pH 5.5 and does not rise again until pH 9 is reached. As shown by Lloyd (1920) there is very little change in the swelling of gelatin between a hydrogen ion concentration of pH 4.5 and 10.5. In a study of the surface tension of ash-free gelatin, Davis, Salisbury and Harvey (1924) report that when the hydrogen ion concentration is at approximately pH 3, a maximum is reached. When the hydrogen ion concentration is decreased, the surface tension drops and does not rise appreciably until the hydrogen ion concentration reaches pH 10, when it again increases. These examples are sufficient to show that there is a range, in pH, over which there is very little or no change in properties, either physically or from the standpoint of ionization.

Hardy and Michaelis determined the isoelectric point of a pure protein by observations on the migration of particles in the electrical field. It can also be determined by suspending the protein in water and measuring the hydrogen ion concentration potentiometrically. This method is not very accurate, due to the low solubility of most proteins in water and to the slight buffer value of proteins at their isoelectric point. There are, however, other methods available for this purpose. These are based on the fact that the osmotic pressure, viscosity, swell-

ing, and electrical conductivity are all at a minimum at the isoelectric point. These methods are more convenient but give only the approximate position of the isoelectric point. Even with the use of these methods, it is necessary to measure the hydrogen ion concentration of these protein solutions at the isoelectric point. The method as suggested by Cohn's definition should provide an easy method of determining the isoelectric point of a protein but due to the wide isoelectric range, only a rough approximation is obtained. At present there is no simple, direct method of determining the isoelectric point of a protein.

The isoelectric point of a few proteins is given below to show the range in this value. It is practically impossible to compare the values closely inasmuch as they were obtained by different methods.

The hydrogen ion concentration at the isoelectric point as determined by cataphoresis for some proteins has been found to be:

Gelatin (about 0.5 per cent)	$cH = 2.5 \times 10^{-6}$	(Michaelis and Grineff, 1912)
Albumin (about 0.6 per cent)	$cH = 2.0 \times 10^{-6}$	(Michaelis and Davidsohn, 1911)
Casein (about 0.2 per cent)	$cH = 2.5 \times 10^{-6}$	(Michaelis and Pechstein, 1912)
Haemoglobin	$cH = 1.8 \times 10^{-7}$	(Michaelis and Davidsohn, 1912)

The hydrogen ion concentration at which maximum precipitation of some proteins occurs is:

Serum albumin	$cH = 3.1 \times 10^{-6}$	(Rona and Michaelis, 1910)
Serum globulin	$cH = 3.6 \times 10^{-6}$	(Rona and Michaelis, 1910)
Edestin	$cH = 1.4 \times 10^{-7}$	(Rona and Michaelis, 1910)
Gliadin	$cH = 6.0 \times 10^{-10}$	(Rona and Michaelis, 1910)
Casein	$cH = 2.4 \times 10^{-5}$	(Alleman, 1912)

The hydrogen ion concentration at the isoelectric point expressed in terms of pH for various proteins was found to be:

Fibrin	pH = 7.0	(Wöhlich, 1923)
Gelatin	pH = 4.65	(Michaelis and Nakashima, 1923)
Serum albumin, rabbit	pH = 4.67	(Michaelis and Nakashima, 1923)
Serum albumin, porpoise	pH = 4.65	(Michaelis and Nakashima, 1923)
Serum albumin, turtle	pH = 4.68	(Michaelis and Nakashima, 1923)
Serum albumin, dog	pH = 4.66	(Michaelis and Nakashima, 1923)
Serum albumin, cow	pH = 4.65	(Michaelis and Nakashima, 1923)
Serum albumin, human	pH = 4.68	(Michaelis and Nakashima, 1923)

The imbibition curve of gelatin shows two isoelectric points, one at pH 4.7 and the other at pH 7.7. (Wilson and Kern, 1922 and 1923.) Similar results were obtained by Higley and Mathews (1924) who measured the absorption of light by gelatin sols as a function of the hydrogen ion concentration and found that the curves show two points of minima, one at pH 4.69 and the other at pH 7.65. They suggest

that this second point may be the isoelectric point of the sol form of gelatin. Hitchcock (1924), however, found that the results of viscosity and osmotic pressure measurements of a one per cent gelatin solution containing varying amounts of hydrochloric acid and sodium hydroxide indicate that gelatin at 40° C. retains its isoelectric point at about pH 4.7. It is difficult to explain this behavior unless gelatin is a mixture of two proteins present in more or less equal amounts, one with an isoelectric point at pH 4.7 and the other at pH 7.7.

III. EXPERIMENTAL

The Problem. The problem of this paper naturally divides itself into two parts; A, the preparation and comparative analysis of the several proteins studied, and B, the comparative acid and alkali binding capacities of these proteins.

A. As noted under "Historical," the series of prolamines previously prepared, as well as the chemical analyses are very incomplete. For this reason it seemed desirable to prepare as complete a series of the prolamines as possible in order to ascertain any chemical or physical differences that might be shown by the prolamines of the different cereals, and more specifically between the prolamines of members of a single group, *i.e.*, the wheat group and the maize group.

The problem then presented itself in the preparation of the known prolamines of the common cereals, wheat, *Triticum vulgare;* spelt, *Triticum spelta;* rye, *Secala cereale;* oats, *Avena sativa;* corn, *Zea mays;* sorghum, *Sorghum vulgare;* barley, *Hordeum vulgare;* and kafir, *Andropogon sorghum;* and of the unknown prolamines of the common cereals, einkorn, *Triticum monococcum;* emmer, *Triticum dicoccum;* durum wheat, *Triticum durum;* and teosinte, *Euchlaena mexicana Schrad,* and into the chemical analysis of these proteins. The chemical analyses which appeared most advantageous to carry out are:

1. Elementary analysis of the new proteins.
2. Nitrogen distribution into nine groups as determined by the method of the Van Slyke analysis.
3. Color tests.
4. True amide nitrogen.
5. Free amino nitrogen of the native protein.
6. Free carboxyl groups of the native protein.

B. The acid and alkali binding of the proteins was the second and most important phase of the problem. As has been pointed out in the previous section, there are two principal methods of determining the amount of acid or alkali bound by a protein. Both methods, electrical

conductivity and the potentiometric measurements of the hydrogen ion concentration, were employed.

The theories which have been advanced concerning this combination may be briefly summarized as follows:

1. Proteins bind acid and alkali according to the empirical adsorption formula.
2. Proteins bind acid and alkali stoichiometrically, *i.e.*, by the purely chemical forces of primary valency.

In carrying out experiments on the binding of acid and alkali by proteins, the following methods appeared most likely to yield data which would serve to differentiate the proteins and to yield information as to the mechanics of the reaction involved in acid or alkali binding.

1. The binding of hydrochloric acid as determined by potentiometric methods when the acid concentration varied from 0.003 normal to 0.300 normal and of sodium hydroxide when the alkali concentration varied from 0.005 normal to 0.500 normal, at ordinary temperatures.

2. The binding of hydrochloric sulfuric and phosphoric acid as determined by potentiometric methods when the acid concentration varied from 0.003 normal to 0.300 normal, at ordinary temperatures.

3. The binding of hydrochloric acid as determined by potentiometric methods when the concentration of acid varied from 0.003 normal to 0.300 normal and of sodium hydroxide when the concentration varied from 0.005 normal to 0.500 normal, at 15°, 25° and 35° C.

4. The binding of hydrochloric acid and sodium hydroxide as determined by potentiometric methods when the concentration of acid or alkali varied from 0.0005 normal to 0.0300 normal, at 22° and 35° C.

5. The binding of varying concentrations of hydrochloric acid and sodium hydroxide as determined by conductivity methods.

Nomenclature: The nomenclature of the prolamines is not uniform. The name "gliadin" was first used as a general designation of the whole group of alcohol soluble proteins as well as a specific name for the prolamine from wheat. Osborne (1908) suggested that the name "gliadin" be reserved for the alcohol soluble protein of wheat and that "prolamine" be used as the group name for the alcohol soluble proteins. At the present time, the prolamine from oats, *Avena sativa* and from rye, *Secale cereale* have also been called "gliadin" by certain workers. If we were to follow this precedent, the prolamines from durum wheat, *Triticum durum;* spelt, *Triticum spelta;* emmer, *Triticum dicoccum;* and einkorn, *Triticum monococcum,* would also be "gliadins," thus making a total of seven. This would lead to much confusion. We therefore concur in Osborne's suggestion that "gliadin" be reserved for the prolamine from wheat, *Triticum vulgare.* Accordingly we

suggest that the prolamines which have been prepared from the cereals be named as follows:

Source of Prolamine	Name of Prolamine [5]
Triticum vulgare	gliadin (name already in common use).
Triticum spelta	speltin.
Triticum durum	durumin.
Triticum dicoccum	dicoccumin.
Triticum monococcum	monococcumin.
Secale cereale	secalin.
Avena sativa	sativin.
Hordeum vulgare	hordein (name already in common use).
Zea mays	zein (name already in common use).
Teosinte	teozein.[6]
Kafir [7]	kafirin (name already in common use).
Sorghum [7]	sorghumin.

A. Preparation and Analysis of the Proteins

Method. The proteins were prepared in the usual manner. Detailed description is given for the individual proteins.

Nitrogen was determined by the Kjeldahl method.

Carbon and hydrogen was determined by the conventional combustion method of the organic chemist.

Moisture content was determined by drying at 100° C., in vacuum. These samples were then ashed in platinum at 550° C.

Organic sulfur was determined by the method described by Hoffman and Gortner (1923).

The nitrogen distribution into nine groups was carried out as described by Van Slyke (1911a), except that the acid insoluble humin was filtered off immediately after hydrolysis and its nitrogen content determined. The length of hydrolysis was 24 hours in each instance. The phosphotungstates of the bases were all cooled at approximately the same temperature in an ice-cooled refrigerator. In determining the arginine nitrogen, the apparatus described by Holm (1920) was used.

Gliadin from Triticum vulgare. In preparing gliadin the crude glutin was washed from flour in the usual manner and then extracted with 70 per cent alcohol. The alcoholic solution was centrifuged until

[5] These names are used in this paper to designate the prolamines prepared from the respective cereals.

[6] Teozein was chosen to indicate its possible genetic relationship to the prolamine of *Zea mays* (see "Historical"). The physical and chemical characteristics of the protein (see later) show a remarkable resemblance to zein.

[7] Both belong to the sorghum group, but to different classes. Kafir is a grain sorghum and sorghum is a forage or sweet sorghum. (Ball and Rothgeb, 1918.)

clear and then concentrated to a sirup under diminished pressure. This sirup was poured into cold water containing a trace of sodium chloride. The gliadin separated in a gummy, sticky mass which soon settled to the bottom of the container. This was removed, dissolved in alcohol and again poured into water. The precipitate was washed with water several times by decantation. After removing from the container, the protein was dried (below 60° C.), ground to a fine powder and extracted with absolute alcohol and with ether. This treatment removed a small amount of oil and coloring material. A pure white powder was obtained which was analyzed with the following results:

		Ash and moisture free
	Per cent	Per cent
Moisture	8.40	
Ash	0.45	
Nitrogen	16.22	17.78
Sulfur	0.91	0.99

Table I shows the nitrogen distribution of gliadin. These figures agree very well with those given by Van Slyke and any differences can be attributed to slight modifications in the methods and to unavoidable experimental error.

TABLE I

THE NITROGEN DISTRIBUTION OF GLIADIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average	Van Slyke (1911a)
Ammonia N		24.60	24.63	24.61	25.52
Humin N	insoluble	0.50	0.53	0.52	
	soluble	0.32	0.37	0.35	0.86
Basic N	arginine	6.45	6.31	6.38	5.71
	histidine	5.22	5.60	5.41	5.20
	cystine	1.65	1.71	1.68	1.25
	lysine	0.78	0.36	0.57	0.57
N in filtrate from bases	amino	53.31	53.67	53.49	51.98
	non-amino	6.23	6.04	6.14	8.50
Total		99.06	99.21	99.15	99.77

Speltin from Triticum spelta. Five kilos of spelt was milled to a fine flour and extracted several times with 70 per cent alcohol. The alcoholic solution was centrifuged until clear and concentration to a sirup. The protein was precipitated and purified in the manner described for gliadin. The final product weighed 80 grams or 1.6 per cent of the original seed. It was a powder which had the same appearance as gliadin. The results of the elementary analysis show speltin to contain:

		Ash and moisture free
	Per cent	Per cent
Moisture	4.92	
Ash[a]	1.17	
Carbon	49.12	52.31
Hydrogen	6.61	7.04
Nitrogen	16.04	17.02
Sulfur	1.13	1.20
Oxygen (by difference)		22.43

Table II shows the nitrogen distribution of speltin. It is almost identical with that of gliadin.

TABLE II

THE NITROGEN DISTRIBUTION OF SPELTIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average
Ammonia N		24.09	24.27	24.18
Humin N	insoluble	0.50	0.48	0.49
	soluble	0.52	0.60	0.56
Basic N	arginine	6.43	6.73	6.58
	histidine	4.21	4.66	4.45
	cystine	1.05	0.98	1.02
	lysine	3.53	2.60	3.09
N in filtrate from bases	amino	53.63	53.35	53.49
	non-amino	5.16	5.83	5.50
Total		99.12	99.50	99.36

Durumin from Triticum durum. The gluten was washed out of 10 kilos of patent flour from durum wheat and then extracted several times with 70 per cent alcohol. The alcoholic solution was filtered until clear, concentrated to a sirup and the protein precipitated by pouring this sirup into water. The crude protein was purified as described for gliadin. It appeared very similar to the prolamines from wheat and spelt. The yield was 300 grams or 3 per cent. The final product was a snow-white powder which was analyzed with the following results:

		Ash and moisture free
	Per cent	Per cent
Moisture	5.39	
Ash	0.36	
Carbon	51.15	54.27
Hydrogen	6.23	6.61
Nitrogen	16.42	17.53
Sulfur	0.93	0.99
Oxygen (by difference)		20.60

[a] Although the ash content of some of the proteins is quite appreciable, the amount of *soluble ash* is in every case very low as shown by the specific conductivity of protein plus water. Most of the ash is porcelain dust which was derived from the porcelain ballmill. In some instances it was necessary to grind some of the proteins for many hours in order to reduce them to a sufficiently fine state of division.

clear and then concentrated to a sirup under diminished pressure. This sirup was poured into cold water containing a trace of sodium chloride. The gliadin separated in a gummy, sticky mass which soon settled to the bottom of the container. This was removed, dissolved in alcohol and again poured into water. The precipitate was washed with water several times by decantation. After removing from the container, the protein was dried (below 60° C.), ground to a fine powder and extracted with absolute alcohol and with ether. This treatment removed a small amount of oil and coloring material. A pure white powder was obtained which was analyzed with the following results:

		Ash and moisture free
	Per cent	Per cent
Moisture	8.40	
Ash	0.45	
Nitrogen	16.22	17.78
Sulfur	0.91	0.99

Table I shows the nitrogen distribution of gliadin. These figures agree very well with those given by Van Slyke and any differences can be attributed to slight modifications in the methods and to unavoidable experimental error.

TABLE I

THE NITROGEN DISTRIBUTION OF GLIADIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average	Van Slyke (1911a)
Ammonia N		24.60	24.63	24.61	25.52
Humin N	insoluble	0.50	0.53	0.52	
	soluble	0.32	0.37	0.35	0.86
Basic N	arginine	6.45	6.31	6.38	5.71
	histidine	5.22	5.60	5.41	5.20
	cystine	1.65	1.71	1.68	1.25
	lysine	0.78	0.36	0.57	0.57
N in filtrate from bases	amino	53.31	53.67	53.49	51.98
	non-amino	6.23	6.04	6.14	8.50
Total		99.06	99.21	99.15	99.77

Speltin from Triticum spelta. Five kilos of spelt was milled to a fine flour and extracted several times with 70 per cent alcohol. The alcoholic solution was centrifuged until clear and concentration to a sirup. The protein was precipitated and purified in the manner described for gliadin. The final product weighed 80 grams or 1.6 per cent of the original seed. It was a powder which had the same appearance as gliadin. The results of the elementary analysis show speltin to contain:

TABLE IV

THE NITROGEN DISTRIBUTION OF DICOCCUMIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average
Ammonia N		23.92	23.86	23.89
Humin N	insoluble	0.60	0.56	0.58
	soluble	0.30	0.40	0.35
Basic N	arginine	8.13	7.84	7.99
	histidine	2.72	2.58	2.65
	cystine	1.69	1.45	1.57
	lysine	2.88	3.02	2.95
N in filtrate from bases	amino	53.51	53.79	53.65
	non-amino	6.50	6.52	6.51
Total		100.25	100.02	100.14

concentrated to a sirup. The protein was precipitated and purified in the usual manner. The final product was a light gray powder. The yield was 91 grams or 1.82 per cent of the original seed.

The results of the elementary analysis show:

		Ash and moisture free
	Per cent	Per cent
Moisture	6.28	
Ash	0.38	
Carbon	52.03	55.74
Hydrogen	6.42	6.88
Nitrogen	15.57	16.68
Sulfur	1.07	1.14
Oxygen (by difference)		19.56

Again the results of the elementary analysis and the nitrogen distribution, Table V, show a very close resemblance to the chemical composition of the other "wheat series" prolamines.

TABLE V

THE NITROGEN DISTRIBUTION OF MONOCOCCUMIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average
Ammonia N		24.53	23.84	24.19
Humin N	insoluble	0.90	0.88	0.89
	soluble	0.64	0.63	0.64
Basic N	arginine	7.53	6.86	7.20
	histidine	4.63	5.69	5.05
	cystine	1.10	1.15	1.13
	lysine	2.65	1.95	2.30
N in filtrate from bases	amino	53.20	52.91	53.05
	non-amino	4.59	4.40	4.50
Total		99.59	98.31	99.05

Secalin from Secale cereale. In preparing secalin, white rye flour was extracted with 70 per cent alcohol. The precipitation from the alcoholic solution and the purification of the crude protein was accomplished in the same manner as described for gliadin. The final product was a white powder, very similar in appearance to gliadin.

This protein contained:

		Ash and moisture free
	Per cent	Per cent
Moisture	2.26	
Ash	0.28	
Nitrogen	16.33	16.75
Sulfur	0.95	0.97

The distribution of the nitrogen into nine groups is shown in Table VI. These values agree quite well with those of gliadin except the "ammonia nitrogen," which is significantly lower in secalin.

TABLE VI

THE NITROGEN DISTRIBUTION OF SECALIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average
Ammonia N		22.39	21.96	22.18
Humin N	insoluble	0.74	0.86	0.80
	soluble	0.39	0.34	0.37
Basic N	arginine	6.59	7.00	6.80
	histidine	6.93	7.49	7.21
	cystine	1.45	1.42	1.44
	lysine	0.26	0.57	0.42
N in filtrate from bases	amino	50.30	49.97	50.14
	non-amino	9.69	9.84	9.77
Total		98.74	99.45	99.13

Sativin from Avena sativa. Sativin was prepared in the following manner: 13.6 kilos of finely ground oats were extracted several times with 70 per cent alcohol, the alcoholic solution was filtered until clear and concentrated to a sirup. The protein was precipitated and purified in the usual manner. The final product consisted of a light, gray powder, which contained:

		Ash and moisture free
	Per cent	Per cent
Moisture	4.85	
Ash	0.44	
Nitrogen	14.27	15.08
Sulfur	1.41	1.48

The nitrogen distribution, Table VII, shows about the same amount of basic nitrogen although a slightly different distribution than the prolamines of the wheat group. Excepting arginine, the differences are not much more than can be attributed to experimental errors. From the results of these analyses, this preparation appears to be identical with that prepared by Osborne (1892) and by Lüers and Siegert (1924) by extracting the ground oats first with water and then with alcohol.

TABLE VII
THE NITROGEN DISTRIBUTION OF SATIVIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average
Ammonia N		22.13	22.27	22.20
Humin N	insoluble	0.72	0.68	0.70
	soluble	0.56	0.53	0.55
Basic N	arginine	8.73	9.12	8.93
	histidine	2.02	2.59	2.31
	cystine	1.84	1.64	1.74
	lysine	1.20	1.20	1.20
N in filtrate from bases	amino	54.20	54.99	54.60
	non-amino	5.99	5.73	5.86
Total		97.39	98.75	98.09

Hordein from Hordeum vulgare. In preparing hordein commercial pearled barley was ground and extracted with 70 per cent alcohol. The alcoholic solution was filtered and concentrated to a sirup. The protein was precipitated and purified by the method described for gliadin. The absolute alcohol and ether removed a large amount (approximately 50 per cent of the crude protein precipitate) of oil, coloring matter, etc. After drying at 60° C. and grinding, a light gray powder was obtained which contained:

		Ash and moisture free
	Per cent	Per cent
Moisture	2.58	
Ash	0.37	
Nitrogen	16.22	16.70
Sulfur	0.74	0.76

The nitrogen distribution is shown in Table VIII. The high histidine nitrogen value is noteworthy. This might be caused by proline being precipitated with the bases and appearing in the histidine fraction as Sandstrom (1924) has shown to be possible. Further evidence of this is found in the fact that the non-amino fraction of the nitrogen of the filtrate from the bases is no higher than in any of the other prolamines while the analysis of hordein for amino acids (see page 218) shows a higher proline content. Whether or not hordein contains a larger

TABLE VIII

THE NITROGEN DISTRIBUTION OF HORDEIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average
Ammonia N		23.40	23.36	23.38
Humin N	insoluble	1.18	0.90	1.04
	soluble	0 38	0.41	0.40
Basic N	arginine	6.82	6.42	6.22
	histidine	9.84	10.88	10.36
	cystine	1.30	1.46	1.38
	lysine	3.08	2.95	3.02
N in filtrate from bases	amino	50.43	50.39	50.41
	non-amino	3.83	3.46	3.65
Total		100.26	100.23	100.26

amount of histidine than the other prolamines can only be determined by actually isolating the basic amino acids by Kossel's method.

Zein from Zea mays. Zein was prepared from "corn gluten," the nitrogenous by-product from corn starch manufacture. The gluten was especially prepared for this study by low temperature drying so as not to denature the proteins. The crude gluten was extracted several times with 70 per cent alcohol, the alcoholic solution filtered and concentrated to a sirup. The protein was precipitated by pouring the sirup into cold distilled water. It separated out in a stringy, plastic mass. After redissolving and again precipitating by pouring the alcoholic solution into water, the protein was dried at a low temperature. This hard, hornlike mass was ground to a powder, extracted with absolute alcohol and with ether, which removed a considerable amount of oil and coloring matter. The final product was a light straw colored powder which contained:

		Ash and moisture free
	Per cent	Per cent
Moisture	2.02	
Ash	0.32	
Nitrogen	14.96	15.33
Sulfur	0.35	0.36

The nitrogen distribution analysis, Table IX, shows a marked difference from the proteins previously described in the amount of nitrogen in the bases. Only about 8 per cent of the total nitrogen was precipitated in this fraction. This is not much more than half the amount found in any of the other prolamines with the exception of teozein, kafirin and sorghumin but even these contain about 10 per cent. The percentage of ammonia nitrogen is lower in zein than in the prolamines of the wheat group.

TABLE IX

THE NITROGEN DISTRIBUTION OF ZEIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average
Ammonia N		17.84	18.28	18.06
Humin N	insoluble	0.64	0.58	0.61
	soluble	0.54	0.52	0.51
Basic N	arginine	3.84	4.00	3.92
	histidine	2.30	2.60	2.45
	cystine	0.96	1.00	0.98
	lysine	1.06	0.72	0.89
N in filtrate from bases	amino	66.19	65.97	66.08
	non-amino	5.73	6.06	5.90
Total		99.10	99.73	99.40

Teozein from Teosinte, ***Euchlaena mexicana,*** **Schrad (annual or Florida).** On account of the close relationship between teosinte and maize (see "Historical"), it was thought that the seed of teosinte might contain a prolamine similar to, if not identical with, zein. It was found that teosinte contained a considerable amount of protein soluble in 70 per cent alcohol.

This protein was prepared by grinding the seed to a fine meal and extracting this several times with 70 per cent alcohol. The alcoholic solution was filtered until clear and then concentrated to a sirup. The protein was precipitated and purified in the usual manner. The absolute alcohol and ether removed a considerable amount of a greenish, oil-like substance. A similarity in the physical properties of teozein and zein was noted during preparation. It appeared to be even more insoluble in alcohol than zein as the protein separated out very readily in a leather-like mass on the sides of the flask when the alcoholic solution was concentrated.

The protein was prepared from two 3-kilo portions (A and B) of the ground meal. The final yield was 110 grams of teozein from A and 125 grams from B or an average of 3.92 per cent of the original seed. The final product was a white powder which analyzed as follows:

	A	B	Ash and moisture free Average	Zein [Chittenden and Osborne (1892)]
	Per cent	Per cent	Per cent	Per cent
Moisture	4.32	4.38		
Ash	0.56	0.55		
Carbon	54.90	54.80	57.67	55.23
Hydrogen	7.20	6.90	7.42	7.26
Nitrogen	15.17	15.09	15.91	16.13
Sulfur	0.56	0.55	0.59	0.60
Oxygen (by difference)			18.41	20.78

The elementary analysis shows teozein to be very similar to zein. Both are characterized by a high carbon content. As previously stated, the nitrogen distribution, Table X, shows a resemblance to that of zein, although the basic nitrogen is less in the case of zein.

TABLE X

The Nitrogen Distribution of Teozein as Determined by the Van Slyke Analysis, Expressed in Per Cent of Total Nitrogen

		A	B	Average
Ammonia N		18.95	19.02	18.99
Humin N	insoluble	0.71	0.66	0.69
	soluble	0.46	0.53	0.50
Basic N	arginine	3.76	4.04	3.90
	histidine	3.57	3.58	3.58
	cystine	0.93	0.67	0.80
	lysine	2.86	2.27	2.57
N in filtrate from bases	amino	63.61	65.22	64.44
	non-amino	3.70	3.54	3.62
Total		98.55	99.53	99.09

Kafirin from Kafir (*Andropogon sorghum*). This protein was prepared as described by Johns and Brewster (1916). Kafir seed was ground to a fine meal and extracted several times with 70 per cent alcohol at 70-80° C. The alcoholic solution was filtered and concentrated to a sirup. The protein was precipitated and purified in the usual manner. Kafirin differs from the other prolamines prepared in that it is almost insoluble in cold 70 per cent alcohol but very soluble in hot alcohol. When the concentrated alcoholic solution was poured into water, the protein separated in a stringy, fibrous mass. The final product was a light gray powder which contained:

		Ash and moisture free
	Per cent	Per cent
Moisture	4.13	
Ash	1.11	
Nitrogen	15.06	15.75
Sulfur	0.49	0.52

The nitrogen distribution for kafirin, Table XI, more closely resembles that of zein and teozein than of the prolamines from the other types of cereals. The non-amino nitrogen value for the filtrate from the bases is noteworthy, apparently indicating the practical absence of proline.

Sorghumin from Sorghum Seed (*Sorghum vulgare*), **var. "Early Amber."** This protein was prepared in the same manner as kafirin. Ten kilos of sorghum seed were ground to a fine meal and extracted

TABLE XI

THE NITROGEN DISTRIBUTION OF KAFIRIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average
Ammonia N		20.90	20.63	20.76
Humin N	insoluble	0.69	0.69	0.69
	soluble	0.57	0.75	0.66
Basic N	arginine	4.23	3.62	3.92
	histidine	0.86	2.57	1.71
	cystine	1.15	1.32	1.23
	lysine	2.72	2.23	2.48
N in filtrate from bases	amino	69.07	68.62	68.85
	non-amino	0.26	0.38	0.32
Total		100.45	100.81	100.62

several times with hot, 70 per cent alcohol. This alcoholic solution was filtered and concentrated to a sirup. The protein was precipitated and purified in the usual manner. The absolute alcohol and ether removed a large amount of oil and red anthocyanin. The yield was 140 grams or 1.4 per cent of a reddish-brown powder. The elementary analysis shows:

		Ash and moisture free
	Per cent	Per cent
Moisture	3.60	
Ash	0.61	
Carbon	53.00	55.33
Hydrogen	6.60	6.89
Nitrogen	13.86	14.34
Sulfur	0.59	0.61
Oxygen (by difference)		22.83

The low nitrogen content which is much lower than in any of the other prolamines may be due to impurities. It, however, may be characteristic of this protein as Visco (1921) reported the nitrogen content as 13.61 per cent. Sorghumin is undoubtedly the most difficult of the prolamines to purify. It is even more insoluble than kafirin in cold, 70 per cent alcohol. A 2 per cent solution of sorghumin in 70 per cent alcohol sets to a more or less rigid gel on cooling.

The nitrogen distribution, table XII, also resembles those of the prolamines from kafir, maize and teosinte. It has the characteristically low basic nitrogen content of these prolamines.

Casein from Cow's Milk. This sample of casein was prepared from fat-free milk in the following manner: the milk was warmed to approximately 35° C. and enough dilute hydrochloric acid added to bring

TABLE XII

THE NITROGEN DISTRIBUTION OF SORGHUMIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average
Ammonia N		18.91	19.00	18.96
Humin N	insoluble	2.73	1.84	2.29
	soluble	0.69	0.89	0.79
Basic N	arginine	5.33	4.33	4.83
	histidine	1.12	1.26	1.19
	cystine	0.89	0.94	0.92
	lysine	3.50	3.39	3.45
N in filtrate from bases	amino	59.55	60.53	60.01
	non-amino	5.59	5.06	5.33
Total		98.31	97.24	97.77

the acidity to pH 4.6. After the casein separated, the whey was drained off and the crude protein washed 8 times with water which had been adjusted, with hydrochloric acid, to pH 4.8. The casein was then suspended in water and enough sodium hydroxide (N/2) added to reduce the hydrogen ion concentration to pH 8.5. This alkaline solution was centrifuged twice through a Sharples laboratory super-centrifuge at about 35,000 r. p. m. which removed all suspended matter. The almost water clear solution was vigorously agitated and enough dilute hydrochloric acid to bring the solution to pH 4.6 was slowly added. After washing the precipitated casein several times with distilled water of pH 4.8, it was again dissolved in sodium hydroxide, enough being added to bring the solution to pH 7.02 as described by Cohn (1922). The alkaline solution was again clarified by the Sharples laboratory super-centrifuge and precipitated as described above. This time the casein precipitated in very fine flakes. It was washed 14 times by decantation with distilled water using about 6 volumes of water to one volume of moist casein for each washing, and dried without the aid of heat. After grinding, it was dried for a short time at 100° C. The final product was a snow-white powder which contained:

		Ash and moisture free
	Per cent	Per cent
Moisture	2.79	
Ash	0.12	
Nitrogen	15.24	15.70
Sulfur	0.81	0.83

The results of the nitrogen distribution are given in Table XIII. It is noted that these values agree very closely with those given by Van Slyke (1910).

TABLE XIII

THE NITROGEN DISTRIBUTION OF CASEIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average	Van Slyke (1910)
Ammonia N		10.13	10.26	10.20	10.43
Humin N	insoluble	0.34	0.34	0.34	3.43
	soluble	1.21	1.13	1.17	
Basic N	arginine	9.39	9.01	9.20	7.51
	histidine	5.91	6.61	6.26	4.24
	cystine	1.08	1.02	1.05	1.95
	lysine	8.34	8.63	8.49	7.86
N in filtrate from bases	amino	54.38	53.86	54.12	55.04
	non-amino	8.60	8.91	8.76	9.51
Total		99.38	99.77	99.59	99.97

Fibrin from Blood. Commercial "fibrin from blood" was purified by dissolving it in dilute sodium hydroxide and filtering through many folds of cheesecloth and precipitating by adding an equivalent amount of hydrochloric acid. The precipitated protein was washed with water to the absence of chlorides and dried at a low temperature. This is the same preparation of fibrin that Holm and Gortner (1920) and Gortner and Norris (1923) used in their "humin" studies. The final product was a light gray powder which contained:

		Ash and moisture free
	Per cent	Per cent
Moisture	9.03	
Ash	0.68	
Nitrogen	14.89	16.49
Sulfur	0.88	0.97

The nitrogen distribution is given in Table XIV. This agrees very well with the values obtained by other workers.

Color Tests. A series of color tests were made on the purified proteins to ascertain whether any differences existed which could be detected by this method. Four tests were made.

1. Millon's test for the para-hydroxy-benzene nucleus: approximately 0.05 gram of the protein was placed in a test tube, 5 cc. of water and 2 drops of Milton's reagent added. The tubes were heated in a boiling water bath for about 5 minutes.

2. Reducing or loosely bound sulfur: 0.10 gram of the protein was placed in a test tube, 10 cc. of 15 per cent sodium hydroxide added and the mixture boiled for 1 minute. After the contents of the tube had cooled somewhat, a few drops of lead acetate were added.

TABLE XIV

THE NITROGEN DISTRIBUTION OF FIBRIN AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

		A	B	Average	Gortner and Norris (1923)
Ammonia N		6.96	6.89	6.93	7.40
Humin N	insoluble	1.40	1.48	1.44	2.07
					0.85*
	soluble	1.56	1.39	1.48	0.99
Basic N	arginine	14.03	14.29	14.16	12.72
	histidine	4.94	3.66	4.30	3.77
	cystine	0.92	0.85	0.89	0.49
	lysine	13.59	14.52	14.06	12.25
N in filtrate from bases	amino	55.63	56.09	55.86	54.36
	non-amino	0.19	0.27	0.23	4.62
Total		99.22	99.44	99.35	99.53

3. The Molish test was carried out in the usual manner.

4. The benzaldehyde test for tryptophane: 0.05 gram of the protein was placed in a test tube, 15 cc. of 20 per cent hydrochloric acid and 2 drops of benzaldehyde added. The tubes were then warmed to about 50° C. for a short time and allowed to stand at room temperature for 24 hours.

The results of these tests are given in Table XV. All gave about the same color for the Millon's test. The four proteins of the maize group with the possible exception of Kafirin are peculiar in that the test for tryptophane is negative in each one. Sativin also gave a negative test for this amino acid. The similarity of the proteins from the same type of cereals is shown in these tests.

TABLE XV

THE RELATIVE VALUES OF THE COLOR TESTS OF THE VARIOUS PROTEINS

Protein	Millon's test	Reducing sulfur	Molish test	Benzaldehyde test
Gliadin	++	++	?	+
Speltin	++	++	?	+
Durumin	++	++	?	+
Dicoccumin	++	++	?	+
Monococcumin	++	++	?	?
Secalin	++	+	+	?
Sativin	++	++	++	none
Hordein	++	+	+	+
Zein	++	?	?	none
Teozein	++	+	+	none
Kafirin	++	?	none	?
Sorghumin	++	++	++	none
Casein	++	?	none	+++
Fibrin	++	?	?	++++

* Phosphotungstic acid humin N.

The True Amide Nitrogen. In the Van Slyke analysis, the ammonia nitrogen is derived from both the amide nitrogen of the mono-amino dicarboxyl acids, glutamic and aspartic acids (glutamine and asparagine before hydrolysis) and from the deaminization of some of the amino groups. Although the ammonia from the latter source varies with length of hydrolysis, it may contribute appreciably to the true amide nitrogen thus increasing this value (Gortner and Holm, 1917a). To determine the true amide nitrogen without appreciable deaminization of the amino groups advantage is taken of the fact that the amide group is hydrolyzed in a very short time when boiled with acid. It was determined for the present series of proteins by boiling exactly one gram of the protein with 20 cc. of 20 per cent hydrochloric acid for 4 hours (see Gortner and Holm, 1917a). The hydrochloric acid was then driven off *in vacuo* on a boiling water bath and the ammonia distilled and collected as in the regular Van Slyke analysis.

The results of the determinations, Table XVI, show that relatively little amino nitrogen is split off as ammonia in the interval between 4 and 24 hours' boiling with 20 per cent hydrochloric acid. In most of

TABLE XVI

THE TRUE ACID AMIDE NITROGEN OF THE VARIOUS PROTEINS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

Protein	A	B	Average	From Van Slyke analysis	Difference
Gliadin	24.48	24.00	24.24	24.61	+ 0.37
Speltin	24.46	24.45	24.46	24.18	— 0.28
Durumin	24.07	24.36	24.22	25.34	+ 1.12
Dicoccumin	23.78	23.82	23.80	23.89	+ 0.09
Monococcumin	23.96	24.01	23.99	24.69	+ 0.70
Secalin	21.98	21.92	21.95	22.18	+ 0.23
Sativin	21.65	22.14	21.90	22.20	+ 0.30
Hordein	20.96	21.49	21.22	23.38	+ 2.16
Zein	17.13	17.37	17.25	18.06	+ 0.81
Teozein	18.51	18.44	18.48	18.99	+ 0.51
Kafirin	20.66	20.85	20.76	20.76	0.00
Sorghumin	18.76	19.14	18.95	18.96	+ 0.01
Casein	9.74	9.50	9.62	10.20	+ 0.58
Fibrin	5.98	6.05	6.02	6.93	+ 0.91

the cases the difference does not exceed the difference between duplicates. There is, however, a definite increase in ammonia due to deaminization of the amino groups inasmuch as all of the differences except two are positive.

Free Amino Groups in Native Proteins. It has long been known that proteins contain basic groups, this assumption being based on the ability of the protein to neutralize acid. The specific nature of these groups was first indicated by Kossel and his coworkers, who showed

that the protamines, which are especially rich in one or more of the hexone bases, formed salts of constant composition with sulfuric acid. In many instances the salts are definitely crystalline.

Goto (1902) found that clupeine, which contains 82.2 per cent of arginine binds approximately one equivalent of sulfuric acid for each mol of arginine but was uncertain which amino group was free to bind the acid. This point was settled by Kossel and Cameron (1912) who showed that it was due to the guanidine group of arginine.

Other evidence, however, indicates that in some proteins, at least, one of the amino groups of lysine is free and furnishes a large part

TABLE XVII

PER CENT OF THE TOTAL NITROGEN OF THE NATIVE PROTEIN EXISTING AS FREE AMINO NITROGEN

	Free amino nitrogen	One-half of lysine nitrogen from Van Slyke analysis	Free amino nitrogen
	Per cent	Per cent	Per cent
Gliadin	1.86	0.29	1.10 [10]
Speltin	1.57	1.53	
Durumin	1.90	0.94	
Dicoccumin	1.79	1.47	
Monococcumin	1.70	1.15	
Secalin	1.91	0.21	
Sativin	1.65	0.60	
Hordein	1.50	1.51	0.60 [11]
Zein	1.19	0.45	0.00 [10, 11]
Teozein	1.02	1.28	
Kafirin	1.26	1.24	
Sorghumin	2.03	1.72	
Casein	6.08	4.25	5.51 [10]
Fibrin	8.95	7.03	

of the free amino nitrogen of the protein. (Skraup and Kass, 1906; Levites, 1909; Van Slyke, 1911; and Kossel and Gawrilow, 1912.) No quantitative relations were ascertained between the lysine content and the free amino nitrogen content of the proteins.

Van Slyke and Birchard (1914) found that by using the Van Slyke amino nitrogen apparatus, they were able to determine quantitatively the free amino nitrogen in proteins. To accomplish this the proteins were dissolved, using acetic acid or sodium carbonate if necessary, in a 2 to 4 per cent solution. The nitrous acid was allowed to react with the protein solution for 30 minutes. The mixture was continuously shaken. From their work they conclude that one of the two amino groups of lysine, the ε-group, exists free in the protein molecule and is quantitatively determined by the nitrous acid method. The α-amino

[10] Van Slyke and Birchard (1914).
[11] Kossel and Gawrilow (1912).

groups which make up the greater part of the amino nitrogen found after complete hydrolysis are bound as peptide linkages in the native proteins. The free amino nitrogen in the present series of proteins was therefore determined using Van Slyke's apparatus. The proteins were dissolved in a dilute solution of sodium hydroxide. From the results, Table XVII, a similarity between the proteins from genetically related cereals is noted.

In most cases the amount of free amino nitrogen is in excess of half of the lysine nitrogen as determined by the Van Slyke analysis. From the modern conception of the structure of proteins there is no

TABLE XVIII

GRAM EQUIVALENTS OF SODIUM HYDROXIDE REQUIRED TO NEUTRALIZE THE FREE CARBOXYL GROUPS IN THE NATIVE PROTEINS IN 80 PER CENT ALCOHOL

	cc. of N/14 NaOH required to neutralize 0.5 gram protein	Gram equivalents of NaOH required to neutralize 1 gram protein
	cc.	$\times 10^{-5}$
Gliadin	1.32	18.86
Speltin	1.45	20.71
Durumin	1.41	20.07
Dicoccumin	1.42	20.29
Monococcumin	1.45	20.71
Secalin	1.54	22.00
Sativin	2.24	32.00
Hordein	1.26	18.00
Zein	1.20	17.14
Teozein	1.13	16.14
Kafirin	1.10	15.71
Sorghumin [13]	1.24	17.71
Casein	6.09	87.00
Fibrin	3.09	44.14

reason for assuming that the free amino group of lysine contributes all of the amino nitrogen of the protein. Felix (1920) states that the assertion that the ε-amino group of lysine is the only amino group free in unhydrolyzed proteins is not justified.

Free Carboxyl Groups in the Native Proteins. It was thought desirable to titrate the native proteins with sodium hydroxide to determine the actual acidity, (*i.e.*, the free carboxyl groups). As has been shown by Foreman (1920) amino acids, in about 85 per cent alcoholic solutions, can be titrated directly with alkali using phenolphthalein as an indicator. In this concentration of alcohol, the amino group displays no basicity to phenolphthalein and any free carboxyl groups can be titrated. In other words amino acids in 85 per cent alcohol are no longer amphoteric but can be titrated with the same ease as other organic acids.

[13] End-point somewhat uncertain due to the color of the alcoholic solution of sorghumin.

that the protamines, which are especially rich in one or more of the hexone bases, formed salts of constant composition with sulfuric acid. In many instances the salts are definitely crystalline.

Goto (1902) found that clupeine, which contains 82.2 per cent of arginine binds approximately one equivalent of sulfuric acid for each mol of arginine but was uncertain which amino group was free to bind the acid. This point was settled by Kossel and Cameron (1912) who showed that it was due to the guanidine group of arginine.

Other evidence, however, indicates that in some proteins, at least, one of the amino groups of lysine is free and furnishes a large part

TABLE XVII

Per Cent of the Total Nitrogen of the Native Protein Existing as Free Amino Nitrogen

	Free amino nitrogen	One-half of lysine nitrogen from Van Slyke analysis	Free amino nitrogen
	Per cent	Per cent	Per cent
Gliadin	1.86	0.29	1.10 [10]
Speltin	1.57	1.53	
Durumin	1.90	0.94	
Dicoccumin	1.79	1.47	
Monococcumin	1.70	1.15	
Secalin	1.91	0.21	
Sativin	1.65	0.60	
Hordein	1.50	1.51	0.60 [11]
Zein	1.19	0.45	0.00 [10, 11]
Teozein	1.02	1.28	
Kafirin	1.26	1.24	
Sorghumin	2.03	1.72	
Casein	6.08	4.25	5.51 [10]
Fibrin	8.95	7.03	

of the free amino nitrogen of the protein. (Skraup and Kass, 1906; Levites, 1909; Van Slyke, 1911; and Kossel and Gawrilow, 1912.) No quantitative relations were ascertained between the lysine content and the free amino nitrogen content of the proteins.

Van Slyke and Birchard (1914) found that by using the Van Slyke amino nitrogen apparatus, they were able to determine quantitatively the free amino nitrogen in proteins. To accomplish this the proteins were dissolved, using acetic acid or sodium carbonate if necessary, in a 2 to 4 per cent solution. The nitrous acid was allowed to react with the protein solution for 30 minutes. The mixture was continuously shaken. From their work they conclude that one of the two amino groups of lysine, the ε-group, exists free in the protein molecule and is quantitatively determined by the nitrous acid method. The α-amino

[10] Van Slyke and Birchard (1914).
[11] Kossel and Gawrilow (1912).

The potential of the hydrogen electrode was then measured by placing the electrode in the series:

$$H_2,\ H^+ \,||\, Hg,\ HgCl,\ KCl\ (\text{Normal})$$

A Leeds and Northrup, Type K potentiometer with a high sensitivity galvanometer and a saturated potassium chloride salt bridge was used to measure the potential of the system.

The hydrogen ion concentration and pH values were obtained from the tables given by Schmidt and Hoagland (1919) who used the well known Nernst formula,

$$\pi = \frac{RT}{nF} \ln \frac{1}{(cH)}$$

for calculating the hydrogen ion concentration.

The amount of acid or alkali bound was calculated as follows: The hydrogen ion concentration of the different concentrations of acid and of alkali used was determined potentiometrically. These values are given in Tables XIX and XX. The ionization curves were then plotted from these data as shown in Figs. 1 and 2. From these graphs, the equilibrium ion concentration can be converted into normality. The only assumption which it is necessary to make in this method of calculation is that the same normality of acid or alkali gives the same hydrogen or hydroxyl ion concentration (*i.e.*, ionizes the same) when protein is present in the solution as when no protein is present. It is readily seen that *the degree of ionization as determined by conductivity methods does not agree with the values obtained by potentiometric methods. In the case of hydrochloric acid there is relatively little difference but in the case of sodium hydroxide, the difference is much greater. In a 0.5 N solution, 83 per cent ionization being indicated by the former method as compared with 50 per cent ionization by the potentiometric method.* The dissociation constants listed in physical chemistry tables are almost invariably those determined by conductivity methods and therefore cannot be used in accurate hydrogen ion concentration studies.

No temperature correction was necessary in this method of calculation as the hydrogen ion concentration was used only to determine the normality of the equilibrium solution. An illustration of the method will make this clear. If we wish to start with N/x acid and find that the equilibrium E.M.F. gives a hydrogen ion concentration of y which, from the chart, corresponds to N/z acid, then the amount of acid bound *n*, is obtained from the formula:

$$n = N/x - N/z.$$

This corresponds to the formula:

$$n = N - \frac{cH}{\alpha}$$

TABLE XIX

THE POTENTIOMETRIC DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF VARYING NORMALITIES OF THE ACID AND ALKALI SOLUTIONS USED IN THIS PAPER

Hydrochloric acid				Sodium hydroxide			
N	E.M.F.	pH	cH	N	E.M.F.	pH	cOH
0.003	430.0	2.485	0.00327	0.005	968.3	11.583	0.00488
0.006	413.8	2.212	0.00616	0.010	985.0	11.868	0.00744
0.009	404.5	2.053	0.00883	0.020	1002.8	12.169	0.0149
0.012	397.0	1.927	0.0118	0.030	1012.0	12.324	0.0214
0.018	387.4	1.765	0.0172	0.040	1019.4	12.450	0.0284
0.024	380.1	1.642	0.0229	0.050	1024.4	12.534	0.0345
0.030	374.8	1.552	0.0281	0.060	1028.9	12.610	0.0412
0.045	365.7	1.398	0.0400	0.080	1035.6	12.723	0.0534
0.060	358.7	1.280	0.0525	0.100	1040.2	12.800	0.0641
0.075	352.1	1.169	0.0679	0.120	1044.4	12.872	0.0755
0.090	347.0	1.082	0.0828	0.140	1048.3	12.935	0.0880
0.105	343.5	1.023	0.0950	0.160	1051.5	12.991	0.0993
0.120	340.4	0.970	0.107	0.200	1057.0	13.085	0.123
0.150	334.9	0.877	0.132	0.250	1062.0	13.170	0.149
0.180	330.4	0.801	0.157	0.300	1065.1	13.222	0.169
0.210	326.3	0.732	0.185	0.350	1068.5	13.270	0.189
0.240	322.0	0.659	0.219	0.400	1070.9	13.320	0.211
0.270	319.3	0.614	0.243	0.450	1073.2	13.358	0.231
0.300	317.0	0.575	0.266	0.500	1075.0	13.389	0.248

TABLE XIX—*Continued*

THE POTENTIOMETRIC DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF VARYING NORMALITIES OF THE ACID AND ALKALI SOLUTIONS USED IN THIS PAPER

Sulfuric acid				Phosphoric acid				Phosphoric acid in 30 per cent alcohol			
N	E.M.F.	pH	cH	N	E.M.F.	pH	cH	N	E.M.F.	pH	cH
0.003	436.3	2.591	0.00256	0.003	435.0	2.570	0.00269	0.003	441.0	2.671	0.00213
0.006	418.5	2.291	0.00512	0.006	421.3	2.338	0.00460	0.006	430.6	2.495	0.00320
0.009	410.0	2.147	0.00712	0.009	413.4	2.205	0.00624	0.009	424.3	2.389	0.00409
0.012	401.7	2.007	0.00984	0.012	406.9	2.094	0.00804	0.012	420.0	2.316	0.00484
0.018	393.3	1.865	0.0137	0.018	399.2	1.964	0.0108	0.018	417.2	2.268	0.00539
0.024	385.5	1.733	0.0186	0.024	394.8	1.890	0.0129	0.024	415.0	2.232	0.00587
0.030	381.1	1.659	0.0220	0.030	391.0	1.826	0.0149	0.030	413.0	2.198	0.00634
0.045	372.6	1.514	0.0306	0.045	385.0	1.724	0.0189	0.045	399.1	1.963	0.0109
0.060	365.2	1.389	0.0408	0.060	380.3	1.645	0.0227	0.060	394.2	1.879	0.0132
0.075	360.5	1.310	0.0490	0.075	375.9	1.570	0.0269	0.075	390.0	1.809	0.0156
0.090	357.5	1.260	0.0551	0.090	372.9	1.519	0.0302	0.090	386.9	1.756	0.0176
0.105	354.0	1.200	0.0631	0.105	370.3	1.476	0.0336	0.105	384.0	1.707	0.0197
0.120	351.0	1.150	0.0709	0.120	368.7	1.449	0.0356	0.120	382.0	1.674	0.0213
0.150	346.0	1.065	0.0861	0.150	365.5	1.395	0.0403	0.150	380.2	1.643	0.0227
0.180	343.0	1.014	0.0968	0.180	362.8	1.349	0.0448	0.180	377.6	1.599	0.0252
0.210	339.6	0.957	0.111	0.210	360.0	1.302	0.0500	0.210	375.0	1.555	0.0278
0.240	336.8	0.910	0.123	0.240	358.2	1.271	0.0535	0.240	373.0	1.521	0.0301
0.270	334.2	0.865	0.136	0.270	356.2	1.237	0.0579	0.270	370.8	1.485	0.0328
0.300	331.6	0.821	0.151	0.300	354.8	1.214	0.0612	0.300	369.0	1.454	0.0352

TABLE XX

THE POTENTIOMETRIC DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF VARYING NORMALITIES OF THE ACID AND ALKALI SOLUTIONS USED IN THIS PAPER (15°)*

Hydrochloric Acid				Sodium hydroxide			
N	E.M.F.	pH	cH	N	E.M.F.	pH	cOH
0.003	431.2	2.505	0.00313	0.005	959.8	11.442	0.00279
0.006	415.5	2.241	0.00576	0.010	983.0	11.834	0.00688
0.009	406.0	2.079	0.00833	0.020	999.3	12.109	0.0130
0.012	398.5	1.952	0.0111	0.030	1008.5	12.265	0.0186
0.018	387.6	1.768	0.0171	0.040	1015.5	12.383	0.0245
0.024	380.0	1.640	0.0230	0.050	1021.4	12.483	0.0308
0.030	375.0	1.555	0.0278	0.060	1024.4	12.534	0.0346
0.045	366.0	1.403	0.0396	0.080	1031.5	12.653	0.0457
0.060	358.0	1.268	0.0540	0.100	1036.2	12.733	0.0548
0.075	351.8	1.164	0.0688	0.120	1040.8	12.811	0.0656
0.090	347.8	1.096	0.0802	0.140	1044.0	12.865	0.0743
0.105	344.6	1.041	0.0909	0.160	1047.9	12.931	0.0867
0.120	342.0	0.997	0.1009	0.200	1052.7	13.012	0.1038
0.150	335.7	0.891	0.129	0.250	1058.6	13.112	0.131
0.180	331.8	0.825	0.150	0.300	1063.0	13.186	0.155
0.210	327.0	0.744	0.180	0.350	1066.4	13.244	0.179
0.240	322.6	0.669	0.214	0.400	1069.4	13.295	0.199
0.270	320.0	0.626	0.237	0.450	1072.1	13.340	0.221
0.300	317.8	0.589	0.258	0.500	1074.0	13.372	0.238

* See text, p. 289.

TABLE XX—*Continued*

THE POTENTIOMETRIC DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF VARYING NORMALITIES OF THE ACID AND ALKALI SOLUTIONS USED IN THIS PAPER (25°)

Hydrochloric Acid				Sodium hydroxide			
N	E.M.F.	pH	cH	N	E.M.F.	pH	cOH
0.003	425.7	2.413	0.00388	0.005	964.0	11.513	0.00330
0.006	413.5	2.206	0.00622	0.010	981.4	11.807	0.00646
0.009	404.1	2.047	0.00898	0.020	998.3	12.092	0.0128
0.012	397.2	1.930	0.0117	0.030	1014.0	12.358	0.0231
0.018	386.8	1.755	0.0176	0.040	1021.5	12.485	0.0309
0.024	379.0	1.623	0.0238	0.050	1024.7	12.539	0.0350
0.030	373.8	1.535	0.0292	0.060	1031.8	12.659	0.0462
0.045	364.0	1.369	0.0428	0.080	1037.0	12.747	0.0565
0.060	356.8	1.248	0.0564	0.100	1041.1	12.821	0.0671
0.075	349.8	1.130	0.0744	0.120	1044.5	12.874	0.0758
0.090	345.0	1.048	0.0895	0.140	1047.3	12.921	0.0846
0.105	341.5	0.988	0.103	0.160	1053.1	13.019	0.105
0.120	337.2	0.916	0.121	0.200	1058.0	13.102	0.128
0.150	331.6	0.821	0.151	0.250	1061.9	13.168	0.149
0.180	327.9	0.759	0.174	0.300	1065.0	13.220	0.168
0.210	323.6	0.686	0.206	0.350	1068.3	13.276	0.191
0.240	319.9	0.624	0.238	0.400	1071.3	13.327	0.215
0.270	318.0	0.592	0.256	0.450	1074.0	13.340	0.238
0.300	315.8	0.555	0.280	0.500	1076.0	13.406	0.258

TABLE XX—*Continued*

THE POTENTIOMETRIC DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF VARYING NORMALITIES OF THE ACID AND ALKALI SOLUTIONS USED IN THIS PAPER (35°)

Hydrochloric Acid				Sodium hydroxide			
N	E.M.F.	pH	cH	N	E.M.F.	pH	cOH
0.003	425.4	2.408	0.00392	0.005	961.0	11.546	0.00357
0.006	413.0	2.198	0.00634	0.010	984.4	11.858	0.00727
0.009	403.1	2.031	0.00934	0.020	1001.9	12.153	0.0144
0.012	396.6	1.920	0.0120	0.030	1011.2	12.310	0.0207
0.018	386.4	1.748	0.0178	0.040	1018.3	12.431	0.0273
0.024	379.0	1.623	0.0238	0.050	1023.6	12.520	0.0336
0.030	374.2	1.541	0.0288	0.060	1027.8	12.592	0.0393
0.045	363.0	1.352	0.0444	0.080	1034.6	12.706	0.0514
0.060	355.8	1.231	0.0591	0.100	1040.2	12.800	0.0641
0.075	348.1	1.101	0.0785	0.120	1044.7	12.877	0.0764
0.090	344.0	1.031	0.0932	0.140	1047.9	12.931	0.0867
0.105	340.4	0.970	0.107	0.160	1050.4	12.973	0.0952
0.120	336.5	0.905	0.125	0.200	1056.3	13.073	0.120
0.150	329.8	0.792	0.161	0.250	1061.1	13.155	0.145
0.180	325.3	0.716	0.192	0.300	1065.2	13.223	0.169
0.210	321.8	0.656	0.222	0.350	1069.0	13.288	0.196
0.240	319.0	0.609	0.246	0.400	1071.8	13.336	0.219
0.270	316.1	0.560	0.276	0.450	1074.9	13.387	0.247
0.300	313.8	0.521	0.302	0.500	1077.3	13.428	0.271

used by former workers for determining n, if their formula is changed to:

$$n = N - \frac{cH}{\alpha'}$$

where N is the original normality, cH is the equilibrium hydrogen ion concentration and α' is the degree of dissociation *as determined potentiometrically*. The relation between the two formulae is readily seen. The only difference being in the method for determining α. Reduced to its simplest form, the formula becomes;

$$n = N - N'$$

where $N' = \frac{cH}{\alpha'}$, the normality of the equilibrium solution.

The values for n show considerable variation especially at the higher concentrations of acid and alkali. It was usually possible to obtain duplicate determinations within $\pm$ one millivolt at the lower concentrations and within ± 0.5 millivolt at the higher concentrations of acid or alkali. These differences do not appear to be very significant but in the case of sodium hydroxide, the original concentration being 0.5 normal, if one sample gave an E.M.F. of 1069.0 millivolts and the other gave 1070.0 millivolts, the equilibrium concentration would be 0.350 and 0.369 normal sodium hydroxide. This would give 150×10^{-4}

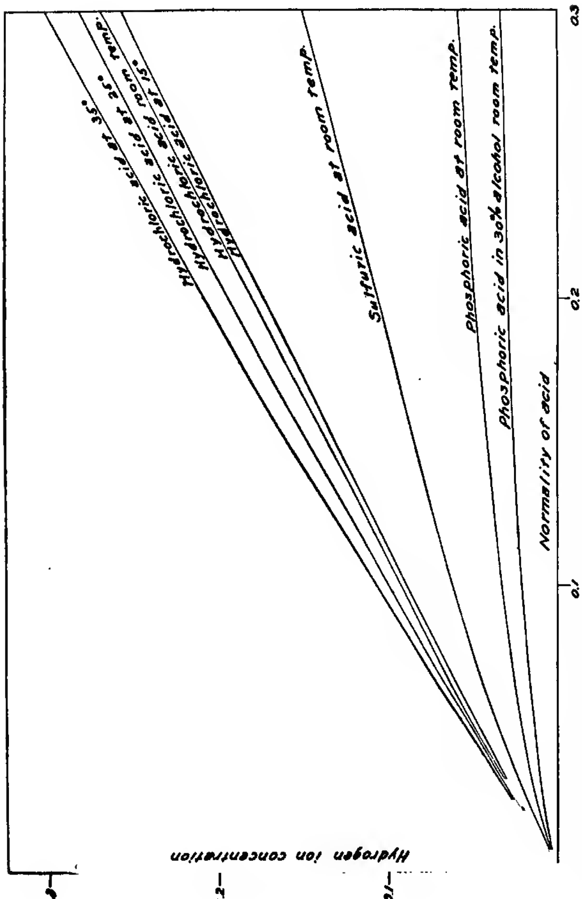


FIG. 1.—The hydrogen ion concentration of varying normalities of hydrochloric, sulfuric and phosphoric acid as measured potentiometrically, including measurements at various temperatures. (Not corrected for the temperature factor of Nernst's formula for reasons explained in text.)

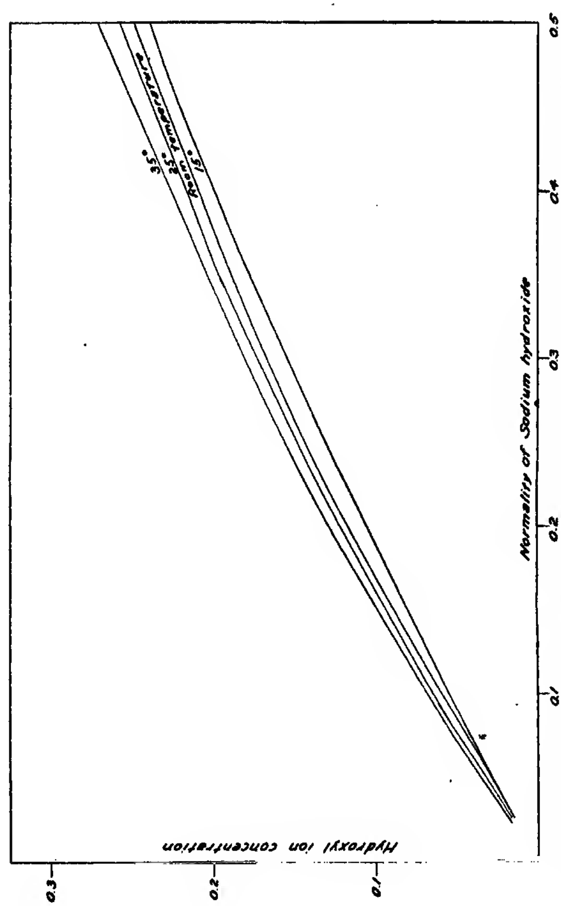


FIG. 2.—The hydroxyl ion concentration of varying normalities of sodium hydroxide as measured potentiometrically, including measurements at various temperatures. (Not corrected for the temperature factor of Nernst's formula for reasons explained in text.)

and 131×10^{-4} gram equivalents of sodium hydroxide bound by one gram of protein, i.e. *an experimental error of* 19×10^{-4} *gram equivalents, although the potentiometric determinations are within the limits allowable for experimental error.* Similar variations are shown by the higher concentrations of acid. Starting with 0.3 normal hydrochloric acid, if one sample gave an E.M.F. of 323 millivolts and the other gave 324 millivolts the equilibrium concentration of hydrochloric acid would be 0.244 and 0.229 normal. This would give 56×10^{-4} and 71×10^{-4} gram equivalents of hydrochloric acid bound by one gram of the protein, an experimental error of 15×10^{-4} gram equivalents. *From these examples it is evident that no conclusions can be drawn from any one determination. It is only the average curve that is significant.* We have accordingly determined a considerable number of points on such curves and base our conclusions on the general slope of the curves rather than on any individual point. The law of averages makes such a curve trustworthy.

The routine method employed for acid binding was to make up a 2 per cent solution of the prolamine in as dilute alcohol as possible (approximately 60 per cent) and use this in making up the solutions as described above. This was very satisfactory in the case of all the prolamines excepting sorghumin. Due to its insolubility in cold alcohol, a 2 per cent solution soon set to a rigid gel. Accordingly, individual samples of this protein as well as casein and fibrin were weighed out. The hydrogen ion concentration of hydrochloric and sulfuric acid in dilute alcohol (less than 30%) is not appreciably different from that in a purely aqueous solution. This does not hold for phosphoric acid, the hydrogen ion concentration being somewhat less in the alcoholic solution. Accordingly, the "ionization" curve for phosphoric acid in 30% alcohol was determined (Table XIX, Fig. 1), and used in our calculations.

There was no detectable change in the amount of acid bound whether or not the protein was completely in solution. Special care was taken to study this point during the casein experiments. When the alcoholic solutions of the prolamines were diluted to less than 30% alcoholic solution, protein precipitated in some instances. This was especially noticed in the stronger solutions of acid. No detectable change in the observed E.M.F. was noted in such cases when they were compared in the other samples to which enough alcohol was added to keep the protein in solution.

When determining the amount of sodium hydroxide bound, the alcoholic solution of the protein was not used, as the equilibrium hydroxyl ion concentration obtained in a 1 per cent protein solution in 30% alcohol was higher than that of an aqueous sodium hydroxide solution of the same normality but containing no protein. The binding of

sodium hydroxide by gliadin in approximately 30 per cent alcohol, Table XXI, is given as an example. It is noted that the hydroxyl ion concentration of the equilibrium solution is greater than could be possible from the sodium hydroxide alone even if it was completely ionized. No explanation can be given for this peculiar behavior. It is possible that some side reaction is taking place. It is our plan to later investigate this phenomenon. It was necessary to weigh out individual

TABLE XXI

POTENTIOMETRIC TITRATION OF GLIADIN (1 PER CENT) IN 30 PER CENT ALCOHOL PLUS VARYING NORMALITIES OF SODIUM HYDROXIDE

N	E.M.F.	pH	cOH
0.005	964.4	11.520	0.00335
0.010	1000.3	12.126	0.0136
0.020	1023.0	12.510	0.0328
0.030	1033.6	12.689	0.0496
0.040	1041.0	12.814	0.0661
0.050	1045.2	12.885	0.0780
0.060	1048.7	12.945	0.0897
0.080	1055.8	13.065	0.118
0.100	1060.5	13.145	0.142
0.120	1065.2	13.223	0.169
0.140	1068.7	13.283	0.194
0.160	1073.0	13.355	0.229
0.200	1078.4	13.447	0.283
0.250	1082.3	13.512	0.330
0.300	1087.3	13.597	0.400
0.350	1089.5	13.634	0.436
0.400	1091.0	13.660	0.462
0.450	1093.2	13.696	0.503
0.500	1095.0	13.727	0.541

samples of the various proteins for determining the alkali binding capacity at the different concentrations of sodium hydroxide.

The experiments were carried out at room temperature, 20-22° C. No attempt was made at an exact temperature control for this set of experiments. This, as will be shown later, may be the cause of some of the variations in the amount of acid or alkali bound.

The results of the determinations of the binding of hydrochloric acid and sodium hydroxide by the 14 proteins are given in Tables XXII to XLIX inclusive. N in the first column is the original normality of the acid or alkali used; the second, E.M.F., is the measured potential of the system in millivolts using a normal calomel electrode; the third, pH, and the fourth, cH or cOH, are obtained from the E.M.F.; the fifth, N′, is the normality of acid or alkali calculated from the cH or cOH, of the equilibrium solution in the manner noted above; and n = N — N′, or the amount of acid or alkali bound by the protein.

TABLE XXII

POTENTIOMETRIC TITRATION OF GLIADIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH	N'	n
—	624.0	5.765	0.00000172	—	—
0.003	465.5	3.084	0.000823	0.001	0.002
0.006	433.0	2.536	0.00291	0.003	0.003
0.009	416.5	2.258	0.00554	0.006	0.003
0.012	407.8	2.110	0.00773	0.008	0.004
0.018	398.2	1.947	0.0112	0.012	0.006
0.024	392.0	1.843	0.0143	0.016	0.008
0.030	385.8	1.728	0.0183	0.021	0.009
0.045	373.0	1.521	0.0301	0.031	0.014
0.060	364.8	1.383	0.0415	0.045	0.015
0.075	360.0	1.302	0.0500	0.054	0.021
0.090	354.1	1.202	0.0630	0.062	0.023
0.105	352.0	1.167	0.0682	0.075	0.030
0.120	349.5	1.124	0.0750	0.082	0.038
0.150	344.8	1.045	0.0902	0.100	0.050
0.180	338.5	0.939	0.115	0.129	0.051
0.210	334.6	0.872	0.134	0.151	0.059
0.240	328.0	0.761	0.174	0.198	0.042
0.270	325.1	0.712	0.194	0.220	0.050
0.300	322.4	0.666	0.215	0.242	0.058

TABLE XXIII

POTENTIOMETRIC TITRATION OF SPELTIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH	N'	n
—	555.0	4.598	0.0000252	—	—
0.003	461.8	3.023	0.000950	0.001	0.002
0.006	430.2	2.488	0.00327	0.004	0.002
0.009	416.7	2.260	0.00552	0.006	0.003
0.012	405.5	2.071	0.00850	0.009	0.003
0.018	392.8	1.857	0.0139	0.014	0.004
0.024	386.8	1.755	0.0177	0.019	0.005
0.030	379.5	1.632	0.0234	0.024	0.006
0.045	369.2	1.457	0.0349	0.036	0.009
0.060	363.0	1.352	0.0444	0.047	0.013
0.075	358.0	1.268	0.0540	0.059	0.016
0.090	354.8	1.214	0.0612	0.067	0.023
0.105	348.6	1.109	0.0778	0.085	0.028
0.120	347.0	1.082	0.0828	0.091	0.029
0.150	339.0	0.947	0.113	0.126	0.024
0.180	337.1	0.915	0.122	0.136	0.044
0.210	334.6	0.872	0.134	0.151	0.059
0.240	330.3	0.799	0.149	0.168	0.072
0.270	327.6	0.754	0.176	0.199	0.071
0.300	324.8	0.707	0.198	0.224	0.076

TABLE XXIV

POTENTIOMETRIC TITRATION OF DURUMIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH	N'	n
—	637.0	5.985	0.104×10^{-5}	—	—
0.003	459.6	2.985	0.00100	0.001	0.002
0.006	434.8	2.567	0.00271	0.003	0.003
0.009	422.8	2.364	0.00433	0.005	0.004
0.012	411.7	2.176	0.00669	0.007	0.005
0.018	399.1	1.963	0.0109	0.011	0.007
0.024	393.0	1.860	0.0138	0.015	0.009
0.030	384.0	1.707	0.0196	0.020	0.010
0.045	373.1	1.523	0.0300	0.031	0.014
0.060	364.0	1.369	0.0427	0.045	0.015
0.075	358.1	1.270	0.0537	0.058	0.017
0.090	355.2	1.220	0.0603	0.066	0.024
0.105	349.0	1.116	0.0766	0.085	0.020
0.120	347.2	1.085	0.0822	0.091	0.029
0.150	339.7	0.959	0.110	0.122	0.028
0.180	337.5	0.922	0.120	0.134	0.046
0.210	334.8	0.876	0.133	0.149	0.061
0.240	330.3	0.799	0.158	0.176	0.064
0.270	327.0	0.744	0.180	0.204	0.066
0.300	324.0	0.693	0.203	0.231	0.069

TABLE XXV

POTENTIOMETRIC TITRATION OF DICOCCUMIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH	N'	n
—	606.0	5.461	0.00000346	—	—
0.003	466.7	3.106	0.000784	0.001	0.002
0.006	432.5	2.527	0.00297	0.003	0.003
0.009	413.8	2.212	0.00616	0.006	0.003
0.012	405.2	2.065	0.00860	0.009	0.003
0.018	394.0	1.877	0.0133	0.013	0.005
0.024	385.8	1.738	0.0183	0.019	0.005
0.030	382.4	1.681	0.0209	0.022	0.008
0.045	374.5	1.546	0.0283	0.031	0.014
0.060	364.6	1.379	0.0417	0.045	0.015
0.075	358.6	1.278	0.0527	0.056	0.019
0.090	354.5	1.208	0.0619	0.070	0.020
0.105	348.6	1.109	0.0778	0.082	0.023
0.120	346.0	1.065	0.0861	0.090	0.030
0.150	341.2	0.983	0.1042	0.122	0.038
0.180	338.8	0.944	0.114	0.132	0.048
0.210	334.5	0.870	0.135	0.152	0.058
0.240	330.0	0.795	0.160	0.180	0.060
0.270	326.0	0.727	0.187	0.208	0.062
0.300	322.9	0.674	0.212	0.237	0.063

TABLE XXVI

Potentiometric Titration of Monococcumin (1 Per Cent) with Varying Normalities of Hydrochloric Acid

N	E.M.F.	pH	cH	N'	n
—	551.0	4.531	0.0000295	—	—
0.003	465.3	3.082	0.000829	0.001	0.002
0.006	435.2	2.573	0.00267	0.003	0.003
0.009	420.5	2.324	0.00474	0.005	0.004
0.012	411.5	2.173	0.00673	0.007	0.005
0.018	400.5	1.986	0.0103	0.011	0.007
0.024	391.2	1.829	0.0148	0.015	0.009
0.030	385.6	1.734	0.0183	0.020	0.010
0.045	373.6	1.531	0.0294	0.031	0.014
0.060	364.6	1.379	0.0418	0.043	0.017
0.075	360.5	1.310	0.0490	0.055	0.020
0.090	354.4	1.207	0.0622	0.068	0.022
0.105	350.0	1.133	0.0738	0.080	0.025
0.120	347.4	1.089	0.0816	0.090	0.030
0.150	343.0	1.014	0.0968	0.108	0.042
0.180	339.2	0.950	0.112	0.125	0.055
0.210	334.0	0.862	0.137	0.154	0.056
0.240	330.0	0.795	0.160	0.181	0.059
0.270	326.3	0.732	0.185	0.210	0.060
0.300	322.8	0.673	0.213	0.239	0.061

TABLE XXVII

Potentiometric Titration of Secalin (1 Per Cent) with Varying Normalities of Hydrochloric Acid

N	E.M.F.	pH	cH	N'	n
—	604.0	5.427	0.00000374	—	—
0.003	467.2	3.114	0.000770	0.001	0.002
0.006	435.7	2.582	0.00262	0.003	0.003
0.009	422.6	2.360	0.00437	0.004	0.005
0.012	412.7	2.193	0.00642	0.006	0.006
0.018	400.1	1.980	0.0104	0.011	0.007
0.024	389.6	1.802	0.0158	0.016	0.008
0.030	386.0	1.741	0.0182	0.019	0.011
0.045	377.5	1.597	0.0253	0.026	0.019
0.060	370.0	1.471	0.0338	0.035	0.025
0.075	363.5	1.360	0.0438	0.046	0.029
0.090	355.8	1.231	0.0589	0.065	0.029
0.105	351.8	1.164	0.0687	0.074	0.031
0.120	349.5	1.124	0.0750	0.083	0.037
0.150	345.0	1.048	0.0895	0.099	0.051
0.180	341.5	0.988	0.103	0.114	0.066
0.210	336.0	0.896	0.127	0.142	0.068
0.240	332.0	0.828	0.148	0.161	0.079
0.270	328.5	0.770	0.170	0.192	0.078
0.300	325.1	0.712	0.194	0.220	0.080

TABLE XXVIII

POTENTIOMETRIC TITRATION OF SATIVIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH	N'	n
—	543.0	4.395	0.0000402	—	—
0.003	439.5	2.645	0.00227	0.002	0.001
0.006	422.5	2.359	0.00439	0.004	0.002
0.009	411.0	2.164	0.00686	0.006	0.003
0.012	400.0	1.978	0.0105	0.011	0.001
0.018	393.0	1.860	0.0138	0.015	0.003
0.024	391.4	1.833	0.0145	0.016	0.008
0.030	379.5	1.632	0.0234	0.024	0.008
0.045	371.0	1.488	0.0325	0.034	0.011
0.060	363.8	1.366	0.0431	0.046	0.014
0.075	358.4	1.275	0.0527	0.057	0.018
0.090	353.0	1.183	0.0656	0.071	0.019
0.105	348.8	1.113	0.0772	0.085	0.020
0.120	345.8	1.062	0.0869	0.096	0.024
0.150	342.3	1.002	0.100	0.112	0.030
0.180	338.5	0.938	0.111	0.124	0.056
0.210	334.0	0.862	0.137	0.154	0.056
0.240	331.2	0.813	0.153	0.172	0.068
0.270	326.9	0.742	0.181	0.205	0.065
0.300	323.7	0.688	0.205	0.231	0.069

TABLE XXIX

POTENTIOMETRIC TITRATION OF HORDEIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH	N'	n
—	589.5	5.184	0.00000657	—	—
0.003	468.0	3.127	0.000744	0.001	0.002
0.006	430.5	2.493	0.00321	0.003	0.003
0.009	417.4	2.272	0.00535	0.006	0.003
0.012	405.4	2.070	0.00853	0.008	0.004
0.018	395.8	1.907	0.0124	0.013	0.005
0.024	388.0	1.775	0.0169	0.018	0.006
0.030	381.8	1.671	0.0213	0.022	0.008
0.045	372.0	1.505	0.0313	0.033	0.012
0.060	364.5	1.377	0.0419	0.045	0.015
0.075	361.0	1.319	0.0480	0.052	0.023
0.090	356.8	1.248	0.0565	0.060	0.030
0.105	352.0	1.167	0.0682	0.072	0.033
0.120	347.0	1.082	0.0828	0.090	0.030
0.150	343.0	1.014	0.0968	0.105	0.045
0.180	339.0	0.947	0.113	0.126	0.054
0.210	335.4	0.886	0.130	0.145	0.065
0.240	331.8	0.825	0.150	0.169	0.071
0.270	328.0	0.761	0.174	0.197	0.073
0.300	324.8	0.707	0.197	0.223	0.077

TABLE XXX

POTENTIOMETRIC TITRATION OF ZEIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH	N'	n
—	579.0	5.004	0.00000991	—	—
0.003	444.1	2.724	0.00191	0.002	0.001
0.006	421.3	2.337	0.00461	0.004	0.002
0.009	412.2	2.184	0.00654	0.006	0.003
0.012	408.9	2.128	0.00743	0.007	0.005
0.018	399.5	1.970	0.0107	0.010	0.008
0.024	392.2	1.846	0.0142	0.014	0.010
0.030	388.0	1.775	0.0169	0.018	0.012
0.045	376.7	1.584	0.0261	0.028	0.017
0.060	370.4	1.478	0.0332	0.035	0.025
0.075	364.9	1.384	0.0413	0.045	0.030
0.090	359.9	1.300	0.0501	0.053	0.037
0.105	356.6	1.244	0.0572	0.061	0.044
0.120	352.1	1.168	0.0680	0.072	0.048
0.150	347.1	1.084	0.0826	0.092	0.058
0.180	342.4	1.004	0.0992	0.110	0.070
0.210	336.7	0.908	0.124	0.138	0.072
0.240	332.8	0.842	0.144	0.162	0.078
0.270	327.0	0.744	0.180	0.203	0.067
0.300	321.9	0.657	0.220	0.244	0.056

TABLE XXXI

POTENTIOMETRIC TITRATION OF TEOZEIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH	N'	n
—	664.5	6.449	0.355×10^{-6}	—	—
0.003	453.7	2.886	0.00131	0.001	0.002
0.006	430.4	2.492	0.00323	0.003	0.003
0.009	415.7	2.244	0.00572	0.006	0.003
0.012	406.5	2.088	0.00817	0.008	0.004
0.018	397.0	1.927	0.0118	0.012	0.006
0.024	391.5	1.834	0.0146	0.015	0.009
0.030	385.0	1.724	0.0189	0.020	0.010
0.045	374.5	1.547	0.0284	0.031	0.014
0.060	364.5	1.378	0.0419	0.045	0.015
0.075	360.0	1.302	0.0500	0.053	0.022
0.090	354.5	1.209	0.0619	0.063	0.027
0.105	352.8	1.180	0.0661	0.072	0.033
0.120	350.8	1.147	0.0714	0.079	0.041
0.150	345.5	1.056	0.0878	0.097	0.053
0.180	339.5	0.955	0.111	0.124	0.056
0.210	344.3	0.867	0.135	0.152	0.058
0.240	329.8	0.792	0.162	0.179	0.061
0.270	326.0	0.727	0.188	0.213	0.057
0.300	323.1	0.678	0.210	0.237	0.063

TABLE XXXII

POTENTIOMETRIC TITRATION OF KAFIRIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH	N'	n
—	534.0	4.243	0.0000571	—	—
0.003	443.0	2.705	0.00197	0.002	0.001
0.006	423.2	2.370	0.00427	0.004	0.002
0.009	409.0	2.130	0.00741	0.007	0.002
0.012	401.4	2.002	0.00995	0.010	0.002
0.018	393.8	1.874	0.0134	0.014	0.004
0.024	388.0	1.775	0.0168	0.017	0.007
0.030	379.4	1.630	0.0233	0.024	0.006
0.045	372.3	1.509	0.0309	0.032	0.013
0.060	363.0	1.352	0.0444	0.047	0.013
0.075	357.0	1.251	0.0561	0.060	0.015
0.090	353.8	1.197	0.0636	0.068	0.022
0.105	349.6	1.126	0.0750	0.082	0.023
0.120	344.4	1.038	0.0917	0.099	0.021
0.150	343.1	1.016	0.0964	0.106	0.044
0.180	335.8	0.893	0.128	0.143	0.037
0.210	331.0	0.811	0.154	0.171	0.039
0.240	328.3	0.766	0.172	0.196	0.044
0.270	325.8	0.724	0.189	0.214	0.056
0.300	323.0	0.676	0.211	0.238	0.062

TABLE XXXIII

POTENTIOMETRIC TITRATION OF SORGHUMIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH	N'	n
—	530.0	4.176	0.0000667	—	—
0.003	440.5	2.662	0.00217	0.002	0.001
0.006	420.0	2.316	0.00483	0.005	0.001
0.009	409.8	2.144	0.00719	0.007	0.002
0.012	400.5	1.986	0.0103	0.010	0.002
0.018	392.0	1.843	0.0143	0.014	0.004
0.024	386.0	1.741	0.0182	0.018	0.006
0.030	378.5	1.615	0.0243	0.024	0.006
0.045	370.5	1.480	0.0332	0.034	0.009
0.060	362.5	1.344	0.0453	0.048	0.012
0.075	356.5	1.243	0.0573	0.061	0.014
0.090	352.0	1.167	0.0682	0.073	0.017
0.105	348.5	1.108	0.0779	0.085	0.020
0.120	345.0	1.048	0.0895	0.099	0.021
0.150	341.5	0.988	0.103	0.113	0.037
0.180	335.0	0.879	0.132	0.148	0.032
0.210	329.5	0.786	0.164	0.185	0.025
0.240	327.4	0.751	0.177	0.200	0.040
0.270	325.0	0.710	0.195	0.220	0.050
0.300	322.3	0.664	0.216	0.243	0.057

TABLE XXXIV

POTENTIOMETRIC TITRATION OF CASEIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH_e	N'	n
—	564.6	4.760	0.0000174	—	—
0.003	468.4	3.134	0.000737	0.001	0.002
0.006	458.3	2.963	0.00109	0.002	0.004
0.009	440.5	2.663	0.00218	0.004	0.005
0.012	429.3	2.473	0.00336	0.005	0.007
0.018	405.0	2.062	0.00866	0.010	0.008
0.024	392.1	1.845	0.0143	0.015	0.009
0.030	388.0	1.775	0.0168	0.018	0.012
0.045	375.0	1.555	0.0278	0.030	0.015
0.060	364.2	1.372	0.0424	0.044	0.016
0.075	357.4	1.258	0.0553	0.059	0.016
0.090	352.5	1.174	0.0669	0.071	0.019
0.105	349.1	1.118	0.0763	0.083	0.022
0.120	346.3	1.068	0.0861	0.096	0.024
0.150	339.0	0.947	0.113	0.125	0.025
0.180	337.0	0.913	0.122	0.136	0.044
0.210	333.0	0.845	0.143	0.161	0.049
0.240	331.6	0.821	0.151	0.170	0.070
0.270	326.0	0.727	0.187	0.211	0.059
0.300	322.0	0.659	0.219	0.245	0.055

TABLE XXXV

POTENTIOMETRIC TITRATION OF FIBRIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID

N	E.M.F.	pH	cH	N'	n
—	565.0	4.767	0.0000171	—	—
0.003	476.8	3.277	0.000527	0.001	0.002
0.006	457.8	2.956	0.00112	0.001	0.005
0.009	448.3	2.794	0.00161	0.002	0.007
0.012	439.5	2.646	0.00227	0.003	0.009
0.018	423.5	2.376	0.00422	0.005	0.013
0.024	407.2	2.099	0.00795	0.009	0.015
0.030	396.3	1.915	0.0121	0.013	0.017
0.045	382.8	1.688	0.0206	0.022	0.023
0.060	371.0	1.488	0.0325	0.034	0.026
0.075	367.0	1.420	0.0380	0.040	0.035
0.090	363.5	1.361	0.0436	0.047	0.043
0.105	360.2	1.305	0.0496	0.053	0.052
0.120	353.0	1.183	0.0656	0.072	0.048
0.150	344.8	1.045	0.0902	0.098	0.052
0.180	338.1	0.932	0.117	0.131	0.049
0.210	333.0	0.845	0.143	0.162	0.048
0.240	330.0	0.795	0.160	0.180	0.060
0.270	327.0	0.744	0.180	0.204	0.066
0.300	323.8	0.690	0.205	0.233	0.067

TABLE XXXVI

POTENTIOMETRIC TITRATION OF GLIADIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE

N	E.M.F.	pH	cOH	N'	n
—	624.0	5.765	0.588×10^{-8}	—	—
0.005	948.5	11.251	0.00180	0.002	0.003
0.010	976.4	11.722	0.00535	0.006	0.004
0.020	996.2	12.057	0.0115	0.015	0.005
0.030	1005.6	12.216	0.0166	0.024	0.006
0.040	1011.0	12.307	0.0205	0.028	0.012
0.050	1017.0	12.409	0.0259	0.037	0.013
0.060	1025.6	12.555	0.0363	0.052	0.008
0.080	1031.0	12.645	0.0448	0.067	0.013
0.100	1036.5	12.739	0.0554	0.083	0.017
0.120	1038.3	12.769	0.0597	0.098	0.022
0.140	1041.2	12.817	0.0667	0.104	0.036
0.160	1044.0	12.865	0.0743	0.124	0.036
0.200	1048.5	12.942	0.0887	0.140	0.060
0.250	1053.0	13.017	0.105	0.170	0.080
0.300	1058.0	13.102	0.128	0.214	0.086
0.350	1062.0	13.170	0.149	0.260	0.090
0.400	1064.5	13.212	0.165	0.287	0.113
0.450	1067.8	13.268	0.188	0.343	0.107
0.500	1070.4	13.312	0.207	0.374	0.126

TABLE XXXVII

POTENTIOMETRIC TITRATION OF SPELTIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE

N	E.M.F.	pH	cOH	N'	n
—	555.0	4.598	0.402×10^{-9}	—	—
0.005	932.7	10.984	0.000972	0.001	0.004
0.010	978.7	11.761	0.00585	0.007	0.003
0.020	999.0	12.104	0.0129	0.016	0.004
0.030	1004.6	12.199	0.0160	0.024	0.006
0.040	1010.8	12.304	0.0204	0.029	0.011
0.050	1015.8	12.389	0.0248	0.035	0.015
0.060	1020.5	12.467	0.0297	0.042	0.018
0.080	1027.2	12.581	0.0386	0.056	0.024
0.100	1031.2	12.648	0.0451	0.065	0.035
0.120	1036.6	12.740	0.0556	0.083	0.037
0.140	1040.9	12.812	0.0658	0.102	0.038
0.160	1045.2	12.885	0.0779	0.120	0.040
0.200	1052.0	13.000	0.101	0.160	0.040
0.250	1055.9	13.066	0.118	0.185	0.065
0.300	1059.0	13.119	0.133	0.222	0.078
0.350	1062.2	13.172	0.151	0.257	0.093
0.400	1065.9	13.235	0.174	0.300	0.100
0.450	1068.0	13.271	0.189	0.337	0.113
0.500	1070.1	13.307	0.205	0.367	0.133

TABLE XXXVIII

Potentiometric Titration of Durumin (1 Per Cent) with Varying Normalities of Sodium Hydroxide

N	E.M.F.	pH	cOH	N'	n
—	637.0	5.985	0.973×10^{-8}	—	—
0.005	955.0	11.361	0.00232	0.003	0.002
0.010	978.0	11.749	0.00569	0.007	0.003
0.020	997.1	12.073	0.0119	0.016	0.004
0.030	1005.9	12.221	0.0169	0.025	0.005
0.040	1013.0	12.341	0.0222	0.032	0.008
0.050	1019.5	12.452	0.0286	0.040	0.010
0.060	1022.0	12.493	0.0315	0.045	0.015
0.080	1028.0	12.595	0.0398	0.059	0.021
0.100	1032.5	12.670	0.0475	0.073	0.027
0.120	1037.2	12.750	0.0570	0.086	0.034
0.140	1041.9	12.829	0.0685	0.107	0.033
0.160	1046.0	12.899	0.0805	0.126	0.034
0.200	1049.5	12.959	0.0922	0.146	0.054
0.250	1053.5	13.026	0.107	0.171	0.079
0.300	1058.5	13.110	0.131	0.217	0.083
0.350	1062.7	13.181	0.154	0.261	0.089
0.400	1065.7	13.232	0.173	0.303	0.097
0.450	1067.1	13.256	0.183	0.324	0.126
0.500	1070.3	13.310	0.206	0.371	0.129

TABLE XXXIX

Potentiometric Titration of Dicoccumin (1 Per Cent) with Varying Normalities of Sodium Hydroxide

N	E.M.F.	pH	cOH	N'	n
—	606.0	5.461	0.292×10^{-8}	—	—
0.005	951.5	11.301	0.00203	0.002	0.003
0.010	980.2	11.786	0.00618	0.006	0.004
0.020	997.5	12.079	0.0121	0.015	0.005
0.030	1006.8	12.237	0.0175	0.023	0.007
0.040	1010.8	12.304	0.0204	0.030	0.010
0.050	1018.0	12.426	0.0269	0.038	0.012
0.060	1023.4	12.517	0.0333	0.047	0.013
0.080	1029.0	12.612	0.0413	0.060	0.020
0.100	1034.5	12.704	0.0512	0.077	0.023
0.120	1037.0	12.747	0.0565	0.087	0.033
0.140	1039.4	12.788	0.0620	0.097	0.043
0.160	1044.8	12.879	0.0767	0.120	0.040
0.200	1050.4	12.973	0.0952	0.152	0.048
0.250	1055.0	13.051	0.114	0.183	0.067
0.300	1058.9	13.117	0.132	0.220	0.080
0.350	1061.8	13.167	0.148	0.250	0.100
0.400	1064.0	13.203	0.161	0.275	0.125
0.450	1067.2	13.257	0.183	0.317	0.133
0.500	1069.8	13.302	0.202	0.360	0.140

TABLE XL

POTENTIOMETRIC TITRATION OF MONOCOCCUMIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE

N	E.M.F.	pH	cOH	N'	n
—	551.0	4.531	0.343×10^{-9}	—	—
0.005	947.2	11.228	0.00171	0.003	0.002
0.010	977.3	11.737	0.00552	0.007	0.003
0.020	995.0	12.037	0.0110	0.015	0.005
0.030	1005.7	12.218	0.0167	0.023	0.007
0.040	1014.0	12.358	0.0231	0.033	0.007
0.050	1017.8	12.423	0.0267	0.038	0.012
0.060	1022.1	12.495	0.0317	0.045	0.015
0.080	1029.8	12.626	0.0427	0.063	0.017
0.100	1033.7	12.691	0.0497	0.075	0.025
0.120	1040.1	12.799	0.0638	0.098	0.022
0.140	1043.6	12.858	0.0731	0.115	0.025
0.160	1045.5	12.891	0.0789	0.124	0.036
0.200	1050.0	12.967	0.0939	0.149	0.051
0.250	1055.0	13.051	0.114	0.185	0.065
0.300	1059.0	13.119	0.133	0.213	0.087
0.350	1062.1	13.171	0.150	0.255	0.095
0.400	1064.8	13.217	0.167	0.278	0.122
0.450	1067.2	13.257	0.183	0.323	0.127
0.500	1069.8	13.302	0.202	0.363	0.137

TABLE XLI

POTENTIOMETRIC TITRATION OF SECALIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE

N	E.M.F.	pH	cOH	N'	n
—	604.0	5.427	0.271×10^{-8}	—	—
0.005	942.5	11.150	0.00143	0.002	0.003
0.010	971.1	11.633	0.00433	0.005	0.005
0.020	989.1	11.937	0.00874	0.012	0.008
0.030	1003.7	12.184	0.0154	0.020	0.010
0.040	1011.0	12.307	0.0205	0.028	0.012
0.050	1018.0	12.426	0.0270	0.040	0.010
0.060	1022.5	12.502	0.0322	0.045	0.015
0.080	1028.5	12.604	0.0403	0.058	0.022
0.100	1033.3	12.684	0.0490	0.075	0.025
0.120	1037.0	12.747	0.0565	0.085	0.035
0.140	1040.0	12.798	0.0635	0.093	0.047
0.160	1044.1	12.867	0.0746	0.118	0.042
0.200	1048.3	12.938	0.0880	0.136	0.064
0.250	1053.0	13.017	0.105	0.168	0.082
0.300	1056.2	13.071	0.119	0.190	0.110
0.350	1061.0	13.153	0.144	0.245	0.105
0.400	1065.3	13.225	0.170	0.295	0.105
0.450	1067.5	13.262	0.185	0.327	0.123
0.500	1070.0	13.305	0.204	0.370	0.130

TABLE XLII

POTENTIOMETRIC TITRATION OF SATIVIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE

N	E.M.F.	pH	cOH	N'	n
—	543.0	4.395	0.252×10^{-9}	—	—
0.005	947.9	11.240	0.00176	0.002	0.003
0.010	968.7	11.592	0.00396	0.005	0.005
0.020	992.9	12.001	0.01016	0.013	0.007
0.030	1004.4	12.196	0.0158	0.020	0.010
0.040	1010.8	12.304	0.0203	0.028	0.012
0.050	1015.6	12.385	0.0246	0.035	0.015
0.060	1020.7	12.471	0.0300	0.043	0.017
0.080	1028.2	12.598	0.0401	0.059	0.021
0.100	1033.6	12.689	0.0495	0.075	0.025
0.120	1036.5	12.738	0.0554	0.087	0.033
0.140	1041.0	12.814	0.0661	0.103	0.037
0.160	1044.0	12.865	0.0743	0.113	0.047
0.200	1049.6	12.960	0.0925	0.145	0.055
0.250	1054.5	13.042	0.112	0.180	0.070
0.300	1059.0	13.119	0.133	0.222	0.078
0.350	1061.0	13.153	0.144	0.245	0.105
0.400	1063.2	13.189	0.156	0.270	0.130
0.450	1066.5	13.245	0.179	0.316	0.134
0.500	1069.6	13.298	0.200	0.359	0.141

TABLE XLIII

POTENTIOMETRIC TITRATION OF HORDEIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE

N	E.M.F.	pH	cOH	N'	n
—	589.5	5.181	0.151×10^{-8}	—	—
0.005	944.9	11.190	0.00156	0.002	0.003
0.010	970.5	11.623	0.00424	0.005	0.005
0.020	992.0	11.986	0.00983	0.014	0.006
0.030	1002.0	12.155	0.0145	0.020	0.010
0.040	1010.0	12.290	0.0197	0.028	0.012
0.050	1015.0	12.375	0.0240	0.035	0.015
0.060	1017.7	12.421	0.0266	0.037	0.023
0.080	1025.9	12.559	0.0367	0.054	0.026
0.100	1031.1	12.647	0.0450	0.067	0.033
0.120	1033.0	12.679	0.0484	0.073	0.047
0.140	1036.0	12.730	0.0544	0.083	0.057
0.160	1041.0	12.814	0.0661	0.103	0.057
0.200	1046.1	12.901	0.0807	0.127	0.073
0.250	1050.8	12.980	0.0965	0.154	0.096
0.300	1055.3	13.056	0.115	0.187	0.113
0.350	1059.0	13.119	0.133	0.222	0.128
0.400	1062.2	13.172	0.151	0.257	0.143
0.450	1064.9	13.218	0.167	0.294	0.156
0.500	1068.0	13.271	0.189	0.338	0.162

TABLE XLIV

POTENTIOMETRIC TITRATION OF ZEIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE

N	E.M.F.	pH	cOH	N'	n
—	579.0	5.004	0.102×10^{-8}	—	—
0.005	946.8	11.222	0.00169	0.002	0.003
0.010	968.7	11.592	0.00396	0.005	0.005
0.020	988.7	11.930	0.00862	0.011	0.009
0.030	1001.4	12.145	0.0141	0.020	0.010
0.040	1008.0	12.257	0.0183	0.028	0.012
0.050	1015.2	12.378	0.0242	0.035	0.015
0.060	1018.5	12.435	0.0275	0.040	0.020
0.080	1026.0	12.561	0.0369	0.054	0.026
0.100	1032.8	12.676	0.0480	0.072	0.028
0.120	1035.5	12.721	0.0533	0.080	0.040
0.140	1039.5	12.789	0.0623	0.098	0.042
0.160	1043.7	12.860	0.0734	0.112	0.048
0.200	1047.7	12.928	0.0860	0.137	0.063
0.250	1052.0	13.000	0.1011	0.172	0.078
0.300	1058.5	13.111	0.130	0.214	0.086
0.350	1062.0	13.170	0.150	0.255	0.095
0.400	1064.8	13.217	0.167	0.293	0.107
0.450	1067.9	13.269	0.188	0.338	0.112
0.500	1070.2	13.308	0.202	0.370	0.130

TABLE XLV

POTENTIOMETRIC TITRATION OF TEOZEIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE

N	E.M.F.	pH	cOH	N'	n
—	664.5	6.449	0.285×10^{-7}	—	—
0.005	950.0	11.276	0.00191	0.002	0.003
0.010	969.5	11.604	0.00408	0.005	0.005
0.020	991.0	11.969	0.00946	0.013	0.007
0.030	1000.0	12.121	0.0134	0.019	0.011
0.040	1008.7	12.268	0.0188	0.026	0.014
0.050	1016.6	12.402	0.0255	0.035	0.015
0.060	1020.0	12.460	0.0291	0.042	0.018
0.080	1027.9	12.593	0.0397	0.055	0.025
0.100	1031.4	12.652	0.0455	0.068	0.032
0.120	1035.0	12.713	0.0522	0.079	0.041
0.140	1042.5	12.840	0.0700	0.110	0.030
0.160	1045.9	12.897	0.0802	0.127	0.033
0.200	1050.0	12.967	0.0939	0.150	0.050
0.250	1055.0	13.051	0.114	0.185	0.065
0.300	1058.9	13.117	0.133	0.222	0.078
0.350	1062.6	13.179	0.153	0.267	0.083
0.400	1065.0	13.220	0.168	0.295	0.105
0.450	1067.3	13.259	0.184	0.327	0.123
0.500	1069.5	13.296	0.199	0.348	0.152

TABLE XLVI

POTENTIOMETRIC TITRATION OF KAFIRIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE

N	E.M.F.	pH	cOH	N'	n
—	534.0	4.243	0.177×10^{-9}	—	—
0.005	952.5	11.318	0.00211	0.003	0.002
0.010	974.2	11.685	0.00492	0.006	0.004
0.020	991.2	11.972	0.00953	0.013	0.007
0.030	1000.6	12.131	0.0137	0.019	0.011
0.040	1009.2	12.274	0.0191	0.027	0.013
0.050	1012.1	12.326	0.0214	0.030	0.020
0.060	1015.8	12.389	0.0248	0.035	0.025
0.080	1023.7	12.522	0.0337	0.050	0.030
0.100	1029.2	12.615	0.0416	0.061	0.039
0.120	1034.1	12.698	0.0505	0.076	0.044
0.140	1037.4	12.754	0.0574	0.090	0.050
0.160	1042.0	12.831	0.0687	0.107	0.053
0.200	1046.3	12.904	0.0814	0.128	0.072
0.250	1051.7	12.955	0.100	0.160	0.090
0.300	1057.3	13.090	0.124	0.203	0.097
0.350	1060.5	13.144	0.141	0.237	0.113
0.400	1063.0	13.186	0.155	0.267	0.133
0.450	1065.3	13.225	0.170	0.295	0.155
0.500	1067.2	13.257	0.184	0.325	0.175

TABLE XLVII

POTENTIOMETRIC TITRATION OF SORGHUMIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE

N	E.M.F.	pH	cOH	N'	n
—	530.0	4.176	0.152×10^{-9}	—	—
0.005	935.0	11.022	0.00107	0.002	0.003
0.010	958.7	11.423	0.00268	0.004	0.006
0.020	980.2	11.786	0.00618	0.009	0.011
0.030	997.3	12.076	0.0120	0.017	0.013
0.040	1002.7	12.167	0.0149	0.021	0.019
0.050	1011.6	12.317	0.0210	0.029	0.021
0.060	1015.2	12.378	0.0242	0.033	0.027
0.080	1023.7	12.522	0.0337	0.050	0.030
0.100	1026.8	12.575	0.0380	0.056	0.044
0.120	1032.0	12.662	0.0466	0.070	0.050
0.140	1035.2	12.716	0.0526	0.080	0.060
0.160	1041.3	12.819	0.0669	0.103	0.057
0.200	1045.0	12.882	0.0773	0.120	0.080
0.250	1050.2	12.969	0.0945	0.153	0.097
0.300	1056.1	13.070	0.119	0.197	0.103
0.350	1059.0	13.119	0.133	0.220	0.130
0.400	1062.6	13.179	0.152	0.259	0.141
0.450	1064.5	13.211	0.165	0.285	0.165
0.500	1067.0	13.254	0.182	0.323	0.177

TABLE XLVIII

Potentiometric Titration of Casein (1 Per Cent) with Varying Normalities of Sodium Hydroxide

N	E.M.F.	pH	cOH	N'	n
—	564.6	4.760	0.583×10^{-9}	—	—
0.005	795.0	8.656	0.458×10^{-5}	0.000	0.005
0.010	885.0	10.177	0.000152	0.000	0.010
0.020	952.5	11.318	0.00211	0.003	0.017
0.030	987.4	11.908	0.00817	0.010	0.020
0.040	992.0	11.986	0.00983	0.013	0.027
0.050	1011.1	12.309	0.0206	0.028	0.022
0.060	1016.6	12.402	0.0255	0.035	0.025
0.080	1024.2	12.530	0.0344	0.049	0.031
0.100	1030.6	12.638	0.0441	0.063	0.037
0.120	1034.2	12.698	0.0506	0.077	0.043
0.140	1038.2	12.769	0.0592	0.091	0.049
0.160	1041.6	12.824	0.0676	0.105	0.055
0.200	1050.2	12.969	0.0945	0.153	0.047
0.250	1053.6	13.027	0.108	0.173	0.077
0.300	1057.0	13.085	0.123	0.204	0.096
0.350	1061.2	13.156	0.145	0.245	0.105
0.400	1063.7	13.198	0.159	0.275	0.125
0.450	1067.0	13.254	0.182	0.320	0.130
0.500	1069.7	13.300	0.202	0.362	0.138

TABLE XLIX

Potentiometric Titration of Fibrin (1 Per Cent) with Varying Normalities of Sodium Hydroxide

N	E.M.F.	pH	cOH	N'	n
—	570.0	4.852	0.718×10^{-9}	—	—
0.005	852.3	9.624	0.0000427	0.000	0.005
0.010	934.0	11.005	0.00103	0.002	0.008
0.020	970.4	11.621	0.00423	0.006	0.014
0.030	993.6	12.013	0.0104	0.013	0.017
0.040	1005.0	12.206	0.0162	0.023	0.017
0.050	1012.6	12.334	0.0219	0.031	0.019
0.060	1016.8	12.406	0.0257	0.034	0.026
0.080	1025.5	12.553	0.0361	0.053	0.027
0.100	1032.0	12.662	0.0466	0.070	0.030
0.120	1036.4	12.737	0.0552	0.084	0.036
0.140	1040.0	12.798	0.0636	0.098	0.042
0.160	1043.2	12.851	0.0719	0.112	0.048
0.200	1048.5	12.941	0.0887	0.138	0.062
0.250	1053.0	13.017	0.105	0.170	0.080
0.300	1057.3	13.090	0.125	0.207	0.093
0.350	1061.8	13.167	0.148	0.250	0.100
0.400	1063.0	13.186	0.155	0.267	0.133
0.450	1066.8	13.251	0.181	0.318	0.132
0.500	1069.3	13.293	0.198	0.355	0.145

The Binding of Hydrochloric, Sulfuric and Phosphoric Acid by Proteins. Loeb and coworkers have laid much emphasis on the stoichiometric relationship between proteins and acids, contending that the proteins behave like a weak inorganic base when titrated with acid and like a weak inorganic acid when titrated with alkali. They also contend that at the same pH, all acids regardless of their "strength" are bound by proteins in equivalent amounts. Most investigators have found this to hold true in the case of strong acids such as hydrochloric and sulfuric acid. Pauli and Hirschfeld (1914), however, report that in the case of acetic acid, much less acetic than hydrochloric acid is bound at the same normality but when the same strength, based on hydrogen ion concentration, is taken much more acetic than hydrochloric acid is bound.

Experiments were planned in an attempt to determine the amount of various acids bound by proteins. Normal solutions of hydrochloric and sulfuric acid and a molar solution of phosphoric acid were used. This concentration of phosphoric acid was used inasmuch as phosphoric acid ionizes as a strong monovalent acid when the hydrogen ion concentration is greater than 0.251×10^{-4} (below pH 4.6) so that below this pH it requires a molar solution of phosphoric acid to be equivalent to normal hydrochloric or sulfuric acid solutions. (Abbott and Bray, 1909.) Consequently the values of N for phosphoric acid, are actually molar solutions but for the reason given above are considered as normal.

These determinations were carried out as described above, the only difference being that in the case of phosphoric acid, it was necessary to determine α' in 30 per cent alcohol as well as in water alone. These values, as well as for sulfuric acid, are given in Table XIX and Fig. 1.

Due to the fact that the amount of acid bound by all of the proteins was so nearly identical, it was not thought necessary to determine the binding of sulfuric and phosphoric acid by all the proteins. Consequently only two proteins, durumin and casein were used in this set of experiments. The results of these determinations are given in Tables XXIV and XXXIV for hydrochloric acid, and in Tables L to LIII inclusive for sulfuric and phosphorie acid. These results show that on a normality basis, approximately the same amounts of hydrochloric, sulfuric and phosphoric acids are bound by both proteins. Since in the case of phosphoric acid the experimental error in determining the E.M.F. of the equilibrium solution causes a greater error in the value of the amount of acid bound than in the case of hydrochloric or sulfuric acid, the slightly greater binding of phosphorie acid is not especially significant.

TABLE L

POTENTIOMETRIC TITRATION OF DURUMIN (1 PER CENT) WITH VARYING NORMALITIES OF SULFURIC ACID

N	E.M.F.	pH	cH	N'	n
—	637.0	5.985	0.104×10^{-5}	—	—
0.003	464.5	3.068	0.000855	0.001	0.002
0.006	432.4	2.526	0.00298	0.003	0.003
0.009	423.0	2.367	0.00430	0.005	0.004
0.012	413.4	2.205	0.00625	0.007	0.005
0.018	404.3	2.050	0.00891	0.012	0.006
0.024	396.5	1.920	0.0120	0.016	0.008
0.030	391.3	1.831	0.0147	0.019	0.011
0.045	384.0	1.707	0.0197	0.027	0.018
0.060	373.0	1.521	0.0301	0.044	0.016
0.075	369.2	1.457	0.0349	0.052	0.023
0.090	365.0	1.386	0.0411	0.063	0.027
0.105	362.5	1.343	0.0453	0.070	0.035
0.120	359.1	1.287	0.0517	0.080	0.040
0.150	353.4	1.190	0.0646	0.106	0.044
0.180	349.0	1.116	0.0766	0.132	0.048
0.210	345.0	1.048	0.0895	0.160	0.050
0.240	341.3	0.985	0.1034	0.191	0.049
0.270	338.4	0.937	0.116	0.221	0.049
0.300	336.6	0.906	0.124	0.240	0.060

TABLE LI

POTENTIOMETRIC TITRATION OF CASEIN (1 PER CENT) WITH VARYING NORMALITIES OF SULFURIC ACID

N	E.M.F.	pH	cH	N'	n
—	564.7	4.760	0.0000174	—	—
0.003	475.7	3.258	0.000544	0.001	0.002
0.006	448.9	2.804	0.00157	0.002	0.004
0.009	433.5	2.545	0.00286	0.004	0.005
0.012	420.0	2.316	0.00483	0.006	0.006
0.018	404.7	2.058	0.00876	0.012	0.006
0.024	395.0	1.893	0.0128	0.016	0.008
0.030	387.9	1.773	0.0169	0.023	0.007
0.045	377.5	1.598	0.0253	0.035	0.010
0.060	371.0	1.488	0.0325	0.049	0.011
0.075	367.0	1.420	0.0380	0.058	0.017
0.090	362.0	1.336	0.0482	0.074	0.016
0.105	357.5	1.260	0.0551	0.088	0.017
0.120	354.7	1.212	0.0615	0.099	0.021
0.150	350.9	1.148	0.0712	0.120	0.030
0.180	348.3	1.104	0.0788	0.134	0.046
0.210	344.5	1.039	0.0914	0.165	0.045
0.240	341.0	0.980	0.105	0.197	0.043
0.270	338.4	0.937	0.115	0.218	0.052
0.300	335.3	0.884	0.130	0.252	0.048

TABLE LII

POTENTIOMETRIC TITRATION OF DURUMIN (1 PER CENT) WITH VARYING NORMALITIES OF PHOSPHORIC ACID

N	E.M.F.	pH	cH	N'	n
—	637.0	5.985	0.104×10^{-5}	—	—
0.003	481.0	3.347	0.000450	0.001	0.002
0.006	451.8	2.854	0.00139	0.003	0.003
0.009	437.6	2.613	0.00244	0.005	0.004
0.012	432.0	2.519	0.00303	0.007	0.005
0.018	423.8	2.381	0.00418	0.012	0.006
0.024	417.0	2.265	0.00543	0.017	0.007
0.030	413.0	2.198	0.00634	0.020	0.010
0.045	404.9	2.060	0.00869	0.032	0.012
0.060	398.0	1.944	0.0113	0.040	0.020
0.075	394.1	1.878	0.0133	0.051	0.024
0.090	392.0	1.843	0.0143	0.060	0.030
0.105	390.5	1.817	0.0152	0.065	0.040
0.120	389.0	1.792	0.0162	0.073	0.048
0.150	387.7	1.770	0.0170	0.086	0.064
0.180	384.8	1.721	0.0190	0.114	0.066
0.210	381.5	1.666	0.0217	0.140	0.070
0.240	387.6	1.599	0.0252	0.170	0.070
0.270	376.2	1.575	0.0266	0.189	0.081
0.300	374.3	1.543	0.0286	0.217	0.083

TABLE LIII

POTENTIOMETRIC TITRATION OF CASEIN (1 PER CENT) WITH VARYING NORMALITIES OF PHOSPHORIC ACID

N	E.M.F.	pH	cH	N'	n
—	564.6	4.760	0.0000174	—	—
0.003	461.3	3.014	0.000970	0.001	0.002
0.006	446.7	2.767	0.00171	0.003	0.003
0.009	435.8	2.584	0.00261	0.004	0.005
0.012	426.5	2.426	0.00375	0.006	0.006
0.018	419.0	2.299	0.00502	0.007	0.011
0.024	412.5	2.190	0.00644	0.009	0.015
0.030	406.8	2.093	0.00808	0.012	0.018
0.045	396.0	1.910	0.0123	0.021	0.024
0.060	390.3	1.814	0.0154	0.031	0.029
0.075	385.0	1.724	0.0189	0.043	0.032
0.090	380.2	1.643	0.0227	0.059	0.031
0.105	377.6	1.599	0.0252	0.063	0.042
0.120	375.5	1.564	0.0272	0.075	0.045
0.150	371.6	1.498	0.0318	0.100	0.050
0.180	369.0	1.454	0.0352	0.113	0.067
0.210	367.0	1.420	0.0380	0.136	0.074
0.240	364.8	1.383	0.0414	0.150	0.090
0.270	362.7	1.347	0.0449	0.180	0.090
0.300	361.3	1.324	0.0475	0.200	0.100

The Acid and Alkali Binding Capacity of Proteins at 15°, 25° and 35° C. While working on the binding capacity of casein, Van Slyke and Van Slyke (1907) found that at the higher temperatures equilibrium is established in less time but smaller amounts of acid or alkali are bound. This indicates that there is a difference in the amount of acid bound at different temperatures. This point has been overlooked by most investigators and raises the question as to the validity of the conclusions which have been drawn as to the exact amount of acid or alkali bound by a protein.

Experiments were carried out in the same manner as previously described except that they were carried out in a constant temperature room controlled within ± 0.5° C. of the desired temperature. The determinations were made at three temperatures, 15°, 25°, and 35° C. The binding of hydrochloric acid and sodium hydroxide by casein, fibrin, durumin and teozein was determined. The results are shown in Tables LIV to LXXVII inclusive. The calculations were likewise made in the manner previously described. The hydrogen ion concentration of hydrochloric acid and the hydroxyl ion concentration of the sodium hydroxide were given in Table XX and in Figures 1 and 2. Attention is called to the fact that the hydrogen ion concentrations found are greater in some cases than could be possible, assuming 100 per cent ionization. This is due to the fact that no temperature correction was made. For this purpose, the absolute hydrogen ion concentration was not necessary as it is only used as a relative value in calculating the amount of acid or alkali bound and does not enter into the final value of n.

TABLE LIV

POTENTIOMETRIC TITRATION OF DURUMIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID AT 15° C.

N	E.M.F.	pH	cH	N'	n
0.003	452.0	2.857	0.00139	0.001	0.002
0.006	425.0	2.401	0.00398	0.004	0.002
0.009	413.1	2.200	0.00633	0.006	0.003
0.012	403.3	2.034	0.00926	0.009	0.003
0.018	396.1	1.912	0.0122	0.013	0.005
0.024	386.5	1.750	0.0178	0.019	0.005
0.030	381.0	1.657	0.0221	0.023	0.007
0.045	372.0	1.504	0.0313	0.034	0.011
0.060	364.7	1.381	0.0416	0.046	0.014
0.075	359.1	1.287	0.0517	0.060	0.015
0.090	355.0	1.217	0.0607	0.069	0.021
0.105	349.7	1.128	0.0746	0.084	0.021
0.120	345.8	1.062	0.0868	0.096	0.024
0.150	341.0	0.980	0.105	0.122	0.028
0.180	336.7	0.908	0.123	0.144	0.036
0.210	333.6	0.855	0.140	0.164	0.046
0.240	330.0	0.795	0.160	0.186	0.054
0.270	327.8	0.758	0.175	0.205	0.065
0.300	326.0	0.727	0.188	0.219	0.081

TABLE LV

Potentiometric Titration of Teozein (1 Per Cent) with Varying Normalities of Hydrochloric Acid at 15° C.

N	E.M.F.	pH	cH	N'	n
0.003	449.5	2.811	0.00154	0.002	0.001
0.006	429.0	2.468	0.00340	0.004	0.002
0.009	414.5	2.224	0.00598	0.006	0.003
0.012	405.0	2.062	0.00866	0.009	0.003
0.018	392.7	1.855	0.0140	0.015	0.003
0.024	385.5	1.732	0.0185	0.019	0.005
0.030	380.5	1.649	0.0225	0.024	0.006
0.045	371.3	1.493	0.0321	0.035	0.010
0.060	365.7	1.398	0.0400	0.045	0.015
0.075	360.9	1.317	0.0482	0.055	0.020
0.090	355.0	1.217	0.0607	0.068	0.022
0.105	351.2	1.153	0.0704	0.080	0.025
0.120	348.2	1.102	0.0791	0.090	0.030
0.150	342.4	1.004	0.0993	0.115	0.035
0.180	338.0	0.930	0.117	0.135	0.045
0.210	334.5	0.870	0.135	0.158	0.052
0.240	331.0	0.811	0.154	0.178	0.062
0.270	328.6	0.771	0.170	0.199	0.081
0.300	326.9	0.742	0.181	0.213	0.087

TABLE LVI

Potentiometric Titration of Casein (1 Per Cent) with Varying Normalities of Hydrochloric Acid at 15° C.

N	E.M.F.	pH	cH	N'	n
0.003	468.5	3.136	0.000732	0.001	0.002
0.006	446.1	2.758	0.00176	0.002	0.004
0.009	429.0	2.468	0.00340	0.004	0.005
0.012	415.7	2.244	0.00571	0.006	0.006
0.018	400.0	1.978	0.0105	0.011	0.007
0.024	391.0	1.826	0.0149	0.016	0.008
0.030	382.3	1.679	0.0210	0.022	0.008
0.045	373.0	1.521	0.0310	0.033	0.012
0.060	364.5	1.378	0.0417	0.045	0.015
0.075	358.4	1.275	0.0532	0.058	0.017
0.090	354.0	1.200	0.0631	0.071	0.019
0.105	350.8	1.147	0.0715	0.082	0.023
0.120	347.6	1.092	0.0810	0.093	0.027
0.150	342.0	0.997	0.1009	0.113	0.037
0.180	338.0	0.930	0.118	0.138	0.042
0.210	344.5	0.871	0.135	0.158	0.052
0.240	331.1	0.813	0.154	0.181	0.059
0.270	328.4	0.768	0.170	0.199	0.071
0.300	324.5	0.701	0.199	0.233	0.067

TABLE LVII

POTENTIOMETRIC TITRATION OF FIBRIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID AT 15° C.

N	E.M.F.	pH	cH	N'	n
0.003	478.5	3.305	0.000496	0.001	0.002
0.006	459.6	2.985	0.001036	0.001	0.005
0.009	445.5	2.747	0.00180	0.002	0.007
0.012	433.0	2.536	0.00291	0.003	0.009
0.018	417.6	2.275	0.00531	0.006	0.012
0.024	401.7	2.007	0.00984	0.010	0.014
0.030	391.2	1.829	0.0148	0.016	0.014
0.045	377.0	1.589	0.0258	0.028	0.017
0.060	367.4	1.427	0.0373	0.040	0.020
0.075	361.6	1.329	0.0469	0.052	0.023
0.090	355.6	1.227	0.0593	0.067	0.023
0.105	351.0	1.150	0.0709	0.078	0.027
0.120	347.8	1.096	0.0803	0.090	0.030
0.150	342.2	1.000	0.100	0.115	0.035
0.180	338.0	0.930	0.118	0.139	0.041
0.210	333.8	0.859	0.139	0.162	0.048
0.240	330.3	0.799	0.155	0.183	0.057
0.270	327.8	0.758	0.175	0.205	0.065
0.300	325.8	0.724	0.189	0.221	0.079

TABLE LVIII

POTENTIOMETRIC TITRATION OF DURUMIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID AT 25° C.

N	E.M.F.	pH	cH	N'	n
0.003	457.0	2.942	0.00114	0.001	0.002
0.006	433.1	2.538	0.00290	0.003	0.003
0.009	418.7	2.294	0.00508	0.005	0.004
0.012	407.7	2.108	0.00780	0.008	0.004
0.018	394.2	1.879	0.0132	0.013	0.005
0.024	386.0	1.741	0.0182	0.018	0.006
0.030	377.5	1.597	0.0253	0.025	0.005
0.045	366.7	1.415	0.0384	0.038	0.007
0.060	361.0	1.319	0.0480	0.048	0.012
0.075	353.7	1.197	0.0636	0.064	0.011
0.090	348.5	1.107	0.0781	0.078	0.012
0.105	345.0	1.048	0.0895	0.090	0.015
0.120	342.0	0.997	0.1008	0.101	0.019
0.150	336.2	0.899	0.125	0.128	0.022
0.180	331.0	0.811	0.154	0.158	0.022
0.210	327.6	0.754	0.177	0.183	0.027
0.240	324.6	0.703	0.198	0.207	0.033
0.270	322.5	0.667	0.215	0.227	0.043
0.300	318.9	0.607	0.247	0.260	0.040

TABLE LIX

POTENTIOMETRIC TITRATION OF TEOZEIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID AT 25° C.

N	E.M.F.	pH	cH	N'	n
0.003	437.6	2.613	0.00244	0.002	0.001
0.006	419.4	2.306	0.00495	0.005	0.001
0.009	408.0	2.113	0.00771	0.008	0.001
0.012	400.7	1.990	0.0102	0.010	0.002
0.018	390.5	1.818	0.0152	0.015	0.003
0.024	385.2	1.727	0.0188	0.019	0.005
0.030	378.8	1.620	0.0240	0.024	0.006
0.045	368.0	1.437	0.0366	0.037	0.008
0.060	359.4	1.292	0.0511	0.052	0.008
0.075	354.0	1.200	0.0632	0.063	0.012
0.090	350.0	1.133	0.0736	0.075	0.015
0.105	345.8	1.062	0.0869	0.089	0.016
0.120	340.0	0.964	0.109	0.111	0.009
0.150	335.0	0.879	0.132	0.134	0.016
0.180	331.3	0.816	0.152	0.155	0.025
0.210	327.8	0.758	0.174	0.179	0.031
0.240	325.2	0.713	0.194	0.198	0.042
0.270	322.5	0.668	0.214	0.222	0.048
0.300	319.5	0.617	0.241	0.250	0.050

TABLE LX

POTENTIOMETRIC TITRATION OF CASEIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID AT 25° C.

N	E.M.F.	pH	cH	N'	n
0.003	459.7	2.987	0.00104	0.001	0.002
0.006	442.0	2.688	0.00205	0.002	0.004
0.009	428.0	2.451	0.00354	0.004	0.005
0.012	419.0	2.299	0.00502	0.005	0.007
0.018	400.1	1.980	0.0101	0.010	0.008
0.024	389.6	1.802	0.0158	0.016	0.008
0.030	383.4	1.698	0.0201	0.020	0.010
0.045	371.0	1.488	0.0325	0.033	0.012
0.060	363.6	1.362	0.0434	0.044	0.016
0.075	357.0	1.251	0.0561	0.056	0.019
0.090	351.6	1.160	0.0693	0.070	0.020
0.105	346.7	1.077	0.0838	0.084	0.021
0.120	342.7	1.009	0.0990	0.100	0.020
0.150	337.3	0.918	0.121	0.124	0.026
0.180	330.5	0.803	0.157	0.160	0.020
0.210	327.0	0.744	0.180	0.186	0.024
0.240	323.7	0.688	0.205	0.213	0.027
0.270	321.3	0.647	0.225	0.236	0.034
0.300	318.9	0.607	0.247	0.260	0.040

TABLE LXI

POTENTIOMETRIC TITRATION OF FIBRIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID AT 25° C.

N	E.M.F.	pH	cH	N'	n
0.003	471.8	3.192	0.000647	0.001	0.002
0.006	458.6	2.968	0.00108	0.001	0.005
0.009	447.2	2.775	0.00168	0.002	0.007
0.012	437.0	2.603	0.00249	0.003	0.009
0.018	417.5	2.274	0.00533	0.006	0.012
0.024	399.0	1.961	0.0109	0.012	0.012
0.030	391.0	1.826	0.0149	0.016	0.014
0.045	371.0	1.488	0.0325	0.034	0.011
0.060	363.0	1.352	0.0444	0.045	0.015
0.075	355.9	1.232	0.0586	0.059	0.016
0.090	351.0	1.150	0.0709	0.072	0.018
0.105	346.7	1.077	0.0838	0.086	0.019
0.120	341.0	0.980	0.105	0.107	0.013
0.150	335.2	0.882	0.131	0.134	0.016
0.180	330.8	0.808	0.155	0.159	0.021
0.210	327.5	0.752	0.177	0.183	0.027
0.240	324.0	0.693	0.203	0.212	0.028
0.270	321.8	0.656	0.221	0.230	0.040
0.300	319.0	0.609	0.246	0.256	0.044

TABLE LXII

POTENTIOMETRIC TITRATION OF DURUMIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID AT 35° C.

N	E.M.F.	pH	cH	N'	n
0.003	462.0	3.026	0.000942	0.001	0.002
0.006	428.0	2.451	0.00354	0.004	0.002
0.009	414.4	2.222	0.00601	0.006	0.003
0.012	406.5	2.088	0.00817	0.008	0.004
0.018	392.6	1.853	0.0140	0.014	0.004
0.024	385.0	1.724	0.0189	0.020	0.004
0.030	379.1	1.625	0.0237	0.024	0.006
0.045	368.4	1.444	0.0360	0.036	0.009
0.060	359.9	1.300	0.0501	0.050	0.010
0.075	353.2	1.186	0.0651	0.065	0.010
0.090	347.0	1.082	0.0828	0.083	0.007
0.105	344.0	1.031	0.0932	0.094	0.011
0.120	339.8	0.961	0.110	0.111	0.009
0.150	334.0	0.862	0.138	0.138	0.012
0.180	328.0	0.761	0.174	0.170	0.010
0.210	324.3	0.698	0.201	0.196	0.014
0.240	321.8	0.656	0.222	0.216	0.024
0.270	318.5	0.601	0.251	0.250	0.020
0.300	315.5	0.549	0.283	0.281	0.019

TABLE LXIII

Potentiometric Titration of Teozein (1 Per Cent) with Varying Normalities of Hydrochloric Acid at 35° C.

N	E.M.F.	pH	cH	N'	n
0.003	441.7	2.683	0.00207	0.002	0.001
0.006	418.4	2.289	0.00514	0.005	0.001
0.009	409.7	2.142	0.00722	0.007	0.002
0.012	401.0	1.995	0.0101	0.010	0.002
0.018	389.0	1.792	0.0162	0.016	0.002
0.024	380.5	1.648	0.0225	0.022	0.002
0.030	377.0	1.589	0.0258	0.025	0.005
0.045	366.0	1.403	0.0396	0.038	0.007
0.060	357.0	1.251	0.0561	0.054	0.006
0.075	351.0	1.150	0.0709	0.068	0.007
0.090	346.8	1.079	0.0834	0.080	0.010
0.105	343.0	1.014	0.0968	0.093	0.012
0.120	340.4	0.970	0.107	0.104	0.016
0.150	334.7	0.874	0.133	0.130	0.020
0.180	329.5	0.786	0.164	0.158	0.022
0.210	326.0	0.727	0.188	0.183	0.027
0.240	323.0	0.676	0.211	0.207	0.031
0.270	319.1	0.611	0.245	0.241	0.029
0.300	316.5	0.567	0.272	0.268	0.032

TABLE LXIV

Potentiometric Titration of Casein (1 Per Cent) with Varying Normalities of Hydrochloric Acid at 35° C.

N	E.M.F.	pH	cH	N'	n
0.003	468.1	3.129	0.000743	0.001	0.002
0.006	448.5	2.798	0.00159	0.002	0.004
0.009	432.0	2.519	0.00303	0.003	0.006
0.012	420.0	2.316	0.00483	0.005	0.007
0.018	397.3	1.932	0.0117	0.012	0.006
0.024	388.5	1.784	0.0165	0.017	0.007
0.030	381.2	1.660	0.0219	0.022	0.008
0.045	368.5	1.445	0.0359	0.035	0.010
0.060	361.0	1.319	0.0480	0.048	0.012
0.075	353.2	1.186	0.0651	0.065	0.010
0.090	348.9	1.114	0.0769	0.076	0.014
0.105	343.0	1.014	0.0968	0.094	0.011
0.120	340.0	0.964	0.109	0.104	0.015
0.150	334.0	0.862	0.138	0.133	0.017
0.180	328.6	0.771	0.169	0.163	0.017
0.210	324.8	0.707	0.196	0.190	0.020
0.240	321.1	0.644	0.227	0.220	0.020
0.270	318.0	0.592	0.256	0.249	0.021
0.300	315.5	0.549	0.283	0.280	0.020

TABLE LXV

POTENTIOMETRIC TITRATION OF FIBRIN (1 PER CENT) WITH VARYING NORMALITIES OF HYDROCHLORIC ACID AT 35° C.

N	E.M.F.	pH	cH	N'	n
0.003	475.5	3.254	0.000557	0.001	0.002
0.006	463.0	3.043	0.000908	0.001	0.005
0.009	456.0	2.925	0.00119	0.001	0.008
0.012	445.1	2.741	0.00182	0.002	0.010
0.018	420.3	2.321	0.00478	0.005	0.013
0.024	402.0	2.012	0.00973	0.010	0.014
0.030	388.0	1.775	0.0169	0.017	0.013
0.045	375.0	1.555	0.0278	0.027	0.018
0.060	366.5	1.412	0.0388	0.038	0.022
0.075	358.0	1.268	0.0540	0.053	0.022
0.090	352.0	1.167	0.0683	0.067	0.023
0.105	345.4	1.055	0.0882	0.086	0.019
0.120	341.0	0.980	0.105	0.103	0.017
0.150	335.5	0.888	0.125	0.123	0.027
0.180	330.4	0.801	0.157	0.154	0.026
0.210	326.2	0.730	0.186	0.183	0.027
0.240	322.0	0.659	0.219	0.215	0.025
0.270	318.6	0.602	0.250	0.246	0.024
0.300	316.0	0.558	0.277	0.273	0.027

TABLE LXVI

POTENTIOMETRIC TITRATION OF DURUMIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 15° C.

N	E.M.F.	pH	cOH	N'	n
0.005	953.0	11.327	0.00215	0.003	0.002
0.010	980.2	11.786	0.00630	0.008	0.002
0.020	995.6	12.047	0.0113	0.016	0.004
0.030	1005.0	12.206	0.0162	0.023	0.007
0.040	1013.0	12.341	0.0222	0.032	0.008
0.050	1018.0	12.426	0.0270	0.040	0.010
0.060	1022.4	12.500	0.0323	0.048	0.012
0.080	1028.2	12.598	0.0401	0.060	0.020
0.100	1032.0	12.662	0.0466	0.072	0.028
0.120	1036.0	12.730	0.0544	0.088	0.032
0.140	1039.0	12.781	0.0610	0.099	0.041
0.160	1042.1	12.833	0.0690	0.115	0.045
0.200	1049.5	12.958	0.0931	0.155	0.045
0.250	1052.0	13.000	0.1011	0.175	0.075
0.300	1054.8	13.048	0.113	0.203	0.097
0.350	1058.0	13.102	0.128	0.233	0.117
0.400	1061.0	13.153	0.144	0.270	0.130
0.450	1064.2	13.206	0.163	0.313	0.137
0.500	1066.0	13.237	0.175	0.346	0.154

TABLE LXVII

POTENTIOMETRIC TITRATION OF TEOZEIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 15° C.

N	E.M.F.	pH	cOH	N'	n
0.005	950.3	11.281	0.00194	0.003	0.002
0.010	973.3	11.670	0.00474	0.007	0.003
0.020	990.0	11.952	0.00909	0.015	0.005
0.030	1003.0	12.172	0.0150	0.022	0.008
0.040	1010.0	12.290	0.0197	0.030	0.010
0.050	1015.1	12.377	0.0241	0.035	0.015
0.060	1018.4	12.433	0.0274	0.039	0.021
0.080	1023.1	12.512	0.0329	0.055	0.025
0.100	1029.7	12.624	0.0425	0.068	0.032
0.120	1033.0	12.679	0.0484	0.082	0.038
0.140	1036.5	12.739	0.0554	0.091	0.049
0.160	1040.0	12.798	0.0635	0.103	0.057
0.200	1045.0	12.882	0.0773	0.127	0.073
0.250	1050.5	12.975	0.0953	0.162	0.088
0.300	1055.0	13.051	0.114	0.205	0.095
0.350	1058.4	13.109	0.130	0.235	0.115
0.400	1061.0	13.153	0.144	0.268	0.132
0.450	1063.2	13.189	0.156	0.289	0.161
0.500	1065.0	13.220	0.168	0.323	0.177

TABLE LXVIII

POTENTIOMETRIC TITRATION OF CASEIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 15° C.

N	E.M.F.	pH	cOH	N'	n
0.005	858.2	9.724	0.0000537	—	0.005
0.010	876.2	10.028	0.000107	—	0.010
0.020	959.8	11.442	0.00280	0.004	0.016
0.030	988.5	11.927	0.00854	0.012	0.018
0.040	1003.0	12.172	0.0150	0.021	0.019
0.050	1010.0	12.290	0.0197	0.030	0.020
0.060	1015.7	12.387	0.0247	0.037	0.023
0.080	1023.4	12.457	0.0333	0.055	0.025
0.100	1029.6	12.622	0.0423	0.068	0.032
0.120	1035.0	12.713	0.0522	0.090	0.030
0.140	1038.2	12.767	0.0592	0.105	0.035
0.160	1041.1	12.816	0.0664	0.120	0.040
0.200	1045.3	12.887	0.0782	0.140	0.060
0.250	1050.8	12.976	0.0959	0.175	0.075
0.300	1054.0	13.034	0.109	0.210	0.090
0.350	1057.0	13.085	0.123	0.240	0.110
0.400	1060.2	13.139	0.140	0.260	0.140
0.450	1064.1	13.205	0.162	0.300	0.150
0.500	1066.4	13.244	0.178	0.345	0.155

TABLE LXIX

POTENTIOMETRIC TITRATION OF FIBRIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 15° C.

N	E.M.F.	pH	cOH	N'	n
0.005	887.6	10.221	0.000169	—	0.005
0.010	936.7	11.051	0.00114	0.002	0.008
0.020	960.6	11.455	0.00289	0.005	0.015
0.030	989.3	11.940	0.00883	0.013	0.017
0.040	1002.0	12.155	0.0145	0.023	0.017
0.050	1010.0	12.290	0.0198	0.032	0.018
0.060	1014.6	12.368	0.0236	0.041	0.019
0.080	1023.1	12.512	0.0329	0.055	0.025
0.100	1028.9	12.610	0.0411	0.067	0.033
0.120	1032.4	12.669	0.0477	0.080	0.040
0.140	1036.2	12.733	0.0548	0.090	0.050
0.160	1039.2	12.784	0.0615	0.100	0.060
0.200	1044.6	12.875	0.0761	0.125	0.075
0.250	1050.5	12.975	0.0953	0.162	0.088
0.300	1055.4	13.058	0.116	0.205	0.095
0.350	1059.0	13.119	0.133	0.240	0.110
0.400	1061.3	13.158	0.145	0.270	0.130
0.450	1063.2	13.189	0.156	0.289	0.161
0.500	1065.6	13.230	0.172	0.330	0.170

TABLE LXX

POTENTIOMETRIC TITRATION OF DURUMIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 25° C.

N	E.M.F.	pH	cOH	N'	n
0.005	951.7	11.305	0.00204	0.003	0.002
0.010	978.0	11.749	0.00569	0.007	0.003
0.020	990.0	11.952	0.00909	0.014	0.006
0.030	1006.1	12.225	0.0170	0.023	0.007
0.040	1014.6	12.368	0.0236	0.032	0.008
0.050	1020.0	12.460	0.0291	0.040	0.010
0.060	1024.4	12.534	0.0349	0.051	0.009
0.080	1031.0	12.645	0.0448	0.068	0.012
0.100	1036.6	12.740	0.0556	0.083	0.017
0.120	1039.7	12.793	0.0628	0.100	0.020
0.140	1042.6	12.841	0.0703	0.112	0.028
0.160	1045.5	12.890	0.0789	0.125	0.035
0.200	1050.6	12.976	0.0959	0.152	0.048
0.250	1057.5	13.093	0.125	0.208	0.042
0.300	1060.8	13.150	0.143	0.238	0.062
0.350	1064.4	13.210	0.164	0.283	0.067
0.400	1066.5	13.246	0.185	0.325	0.075
0.450	1069.0	13.288	0.196	0.351	0.099
0.500	1071.1	13.324	0.213	0.382	0.118

TABLE LXXI

POTENTIOMETRIC TITRATION OF TEOZEIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 25° C.

N	E.M.F.	pH	cOH	N'	n
0.005	945.0	11.191	0.00157	0.002	0.003
0.010	966.7	11.558	0.00367	0.005	0.005
0.020	984.0	11.851	0.00716	0.012	0.008
0.030	999.8	12.118	0.0133	0.020	0.010
0.040	1007.7	12.252	0.0181	0.027	0.013
0.050	1013.0	12.341	0.0222	0.034	0.016
0.060	1019.2	12.446	0.0282	0.043	0.017
0.080	1026.5	12.570	0.0376	0.057	0.023
0.100	1031.5	12.653	0.0457	0.067	0.033
0.120	1036.0	12.730	0.0544	0.083	0.037
0.140	1040.0	12.798	0.0635	0.098	0.042
0.160	1043.7	12.860	0.0734	0.115	0.045
0.200	1048.3	12.938	0.0880	0.140	0.060
0.250	1052.8	13.014	0.1042	0.170	0.080
0.300	1057.0	13.085	0.123	0.203	0.097
0.350	1061.0	13.153	0.144	0.247	0.103
0.400	1063.4	13.193	0.158	0.275	0.125
0.450	1066.5	13.246	0.179	0.320	0.130
0.500	1069.0	13.288	0.196	0.363	0.137

TABLE LXX11

POTENTIOMETRIC TITRATION OF CASEIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 25° C.

N	E.M.F.	pH	cOH	N'	n
0.005	794.7	8.651	0.00000452	—	0.005
0.010	880.0	10.093	0.000125	—	0.010
0.020	955.5	11.369	0.00237	0.004	0.016
0.030	989.0	11.935	0.00872	0.013	0.017
0.040	1001.0	12.138	0.0139	0.022	0.018
0.050	1009.8	12.283	0.0195	0.030	0.020
0.060	1017.5	12.417	0.0264	0.038	0.022
0.080	1026.0	12.561	0.0368	0.056	0.024
0.100	1032.3	12.665	0.0469	0.073	0.027
0.120	1037.0	12.747	0.0565	0.090	0.030
0.140	1041.7	12.826	0.0679	0.104	0.036
0.160	1044.3	12.870	0.0752	0.120	0.040
0.200	1049.8	12.964	0.0932	0.150	0.050
0.250	1057.0	13.085	0.123	0.200	0.050
0.300	1061.1	13.155	0.144	0.242	0.058
0.350	1064.0	13.203	0.161	0.270	0.080
0.400	1066.2	13.240	0.176	0.305	0.095
0.450	1068.5	13.280	0.192	0.340	0.110
0.500	1071.1	13.324	0.213	0.382	0.118

TABLE LXXIII

POTENTIOMETRIC TITRATION OF FIBRIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 25° C.

N	E.M.F.	pH	cOH	N'	n
0.005	879.0	10.076	0.000120	—	0.005
0.010	931.0	10.955	0.000912	0.001	0.009
0.020	980.0	11.783	0.00614	0.010	0.010
0.030	994.5	12.029	0.0108	0.016	0.014
0.040	1003.5	12.180	0.0153	0.023	0.017
0.050	1013.5	12.349	0.0226	0.034	0.016
0.060	1019.7	12.455	0.0288	0.043	0.017
0.080	1027.0	12.578	0.0383	0.058	0.022
0.100	1030.6	12.638	0.0441	0.065	0.035
0.120	1036.8	12.744	0.0561	0.085	0.035
0.140	1039.8	12.795	0.0630	0.099	0.041
0.160	1043.5	12.856	0.0728	0.114	0.046
0.200	1048.6	12.943	0.0890	0.142	0.058
0.250	1053.6	13.027	0.108	0.176	0.074
0.300	1057.4	13.092	0.125	0.208	0.092
0.350	1061.0	13.153	0.144	0.247	0.103
0.400	1064.2	13.206	0.163	0.283	0.117
0.450	1067.3	13.261	0.185	0.329	0.121
0.500	1070.3	13.310	0.206	0.378	0.122

TABLE LXXIV

POTENTIOMETRIC TITRATION OF DURUMIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 35° C.

N	E.M.F.	pH	cOH	N'	n
0.005	956.0	11.378	0.00242	0.003	0.002
0.010	977.3	11.738	0.00553	0.007	0.003
0.020	997.5	12.079	0.0121	0.016	0.004
0.030	1009.4	12.280	0.0193	0.025	0.005
0.040	1017.0	12.409	0.0259	0.036	0.004
0.050	1022.5	12.502	0.0323	0.043	0.007
0.060	1027.4	12.585	0.0390	0.055	0.005
0.080	1033.4	12.686	0.0492	0.071	0.008
0.100	1038.9	12.781	0.0610	0.090	0.010
0.120	1043.0	12.848	0.0713	0.108	0.012
0.140	1046.2	12.902	0.0810	0.125	0.015
0.160	1049.5	12.958	0.0921	0.145	0.025
0.200	1054.3	13.039	0.111	0.174	0.026
0.250	1059.7	13.131	0.137	0.225	0.025
0.300	1064.4	13.210	0.164	0.282	0.018
0.350	1067.6	13.264	0.186	0.320	0.030
0.400	1070.0	13.305	0.204	0.355	0.045
0.450	1073.0	13.355	0.229	0.393	0.057
0.500	1075.7	13.401	0.255	0.460	0.040

TABLE LXXV

POTENTIOMETRIC TITRATION OF TEOZEIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 35° C.

N	E.M.F.	pH	cOH	N'	n
0.005	947.2	11.228	0.00171	0.002	0.003
0.010	970.3	11.619	0.00421	0.006	0.004
0.020	991.5	11.977	0.00966	0.014	0.006
0.030	1003.4	12.179	0.0152	0.021	0.009
0.040	1013.0	12.341	0.0222	0.033	0.007
0.050	1018.8	12.440	0.0278	0.042	0.008
0.060	1023.5	12.518	0.0334	0.049	0.011
0.080	1031.1	12.647	0.0450	0.066	0.014
0.100	1036.1	12.732	0.0545	0.083	0.017
0.120	1041.7	12.826	0.0678	0.107	0.013
0.140	1045.6	12.892	0.0792	0.123	0.017
0.160	1048.0	12.933	0.0870	0.141	0.019
0.200	1053.9	13.032	0.109	0.175	0.025
0.250	1057.5	13.093	0.126	0.206	0.044
0.300	1062.3	13.174	0.151	0.254	0.046
0.350	1065.6	13.230	0.172	0.296	0.054
0.400	1068.7	13.283	0.194	0.340	0.060
0.450	1071.6	13.332	0.217	0.390	0.060
0.500	1074.6	13.382	0.244	0.438	0.062

TABLE LXXVI

POTENTIOMETRIC TITRATION OF CASEIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 35° C.

N	E.M.F.	pH	cOH	N'	n
0.005	780.2	8.405	0.00000257	—	0.005
0.010	901.0	10.448	0.000283	—	0.010
0.020	967.1	11.565	0.00372	0.005	0.015
0.030	991.0	11.969	0.00946	0.014	0.016
0.040	1006.6	12.233	0.0173	0.028	0.012
0.050	1014.7	12.370	0.0237	0.034	0.016
0.060	1020.5	12.468	0.0297	0.045	0.015
0.080	1028.4	12.602	0.0404	0.061	0.019
0.100	1033.0	12.697	0.0484	0.077	0.023
0.120	1037.9	12.762	0.0585	0.088	0.032
0.140	1043.0	12.848	0.0713	0.112	0.028
0.160	1047.8	12.930	0.0863	0.136	0.024
0.200	1053.5	13.025	0.107	0.172	0.028
0.250	1058.7	13.114	0.132	0.215	0.035
0.300	1063.0	13.186	0.155	0.260	0.040
0.350	1066.5	13.246	0.179	0.308	0.042
0.400	1070.0	13.305	0.204	0.357	0.043
0.450	1073.5	13.363	0.234	0.403	0.047
0.500	1076.0	13.406	0.258	0.465	0.035

TABLE LXXVII

POTENTIOMETRIC TITRATION OF FIBRIN (1 PER CENT) WITH VARYING NORMALITIES OF SODIUM HYDROXIDE AT 35° C.

N	E.M.F.	pH	cOH	N'	n
0.005	867.3	9.878	0.0000760	—	0.005
0.010	928.3	10.909	0.000820	0.001	0.009
0.020	980.0	11.783	0.00612	0.009	0.011
0.030	1003.0	12.172	0.0150	0.020	0.010
0.040	1010.5	12.299	0.0201	0.030	0.010
0.050	1016.5	12.400	0.0254	0.039	0.011
0.060	1021.6	12.486	0.0311	0.047	0.013
0.080	1030.0	12.629	0.0431	0.064	0.016
0.100	1036.0	12.730	0.0544	0.083	0.017
0.120	1040.7	12.809	0.0653	0.104	0.016
0.140	1045.5	12.890	0.0789	0.122	0.018
0.160	1049.7	12.962	0.0928	0.137	0.023
0.200	1053.4	13.024	0.107	0.172	0.028
0.250	1057.8	13.099	0.127	0.207	0.043
0.300	1063.0	13.186	0.155	0.260	0.040
0.350	1066.3	13.242	0.177	0.303	0.047
0.400	1069.0	13.288	0.196	0.343	0.057
0.450	1072.1	13.340	0.221	0.396	0.054
0.500	1075.2	13.392	0.250	0.450	0.050

From the tables it is seen that at 35° C. much less acid or alkali is bound by the protein than at 25° C. while considerably more is bound at 15° C. than at 25° C.

The Binding of Proteins in Dilute Solutions of Acid and Alkali. The acid and alkali used in the experiments described above were of such concentration that even the small initial additions of acid or alkali changed the hydrogen ion concentration of the protein solution through a relatively large range. From the results thus obtained, very little is shown concerning the mode of binding at the lower concentrations of acid or alkali. The question also arises as to the behavior of proteins in very dilute acid and alkali at different temperatures, *i.e.*, whether the logarithmic curves can be correctly exterpolated.

In an attempt to answer these questions, experiments were carried out using more dilute acid and alkali than was employed in the previous determinations. For this purpose a Bovie (1922) titrating vessel was employed. The determinations were made by placing 200 cc. of one per cent protein solution or suspension in the titrating vessel and the desired amount of acid or alkali was added in small increments by means of a 2 cc. burette calibrated in 0.01 cc. intervals. The E.M.F. of the equilibrium mixture was taken after each addition. A Hildebrand bubbling electrode was used. The calculations were made in the same manner as described above. No correction was made for the dilution resulting from the addition of acid or alkali as this only made a three per cent dilution correction at the maximum addition of acid or alkali and is comparable throughout the series. The determinations

were carried out at 22° and 35° C. on durumin, teozein, casein and fibrin.

The results of these titrations are given in Tables LXXVIII to XCIII inclusive. The first column contains the number of cubic centimeters of normal acid or alkali added to 200 cc. of protein solution; the second, the normality of the solution (not corrected for the slight dilution); the third, the E.M.F. as millivolts when a normal calomel electrode was used; the fourth and fifth, the pH and cH or cOH as calculated from the E.M.F.; the sixth, the normality of acid or alkali in the equilibrium solution; the seventh, the amount of acid or alkali bound by the protein; and the eighth, the equivalents of acid or alkali bound per gram of protein.

These results agree quite well with those obtained in the other set of determinations using the Bailey electrode. There is no essential difference in the amounts bound at the different temperatures. This is in agreement with the results of the first two or three additions of acid or alkali in the first set of experiments (Tables XXII to XLIX). In these earlier experiments the amounts of acid or alkali bound did not differ at the different temperatures. It was not until the hydrogen ion concentration was greater than about pH 2.5 or the hydroxyl ion concentration greater than about pH 10.5 that a marked negative temperature coefficient was observed.

TABLE LXXVIII

Electrometric Titration of 200 cc. of 1 Per Cent Durumin Plus Varying Amounts of Hydrochloric Acid at 22° C.

cc. HCl N/1	N	E.M.F.	pH	cH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	——	630.0	5.866	0.00000136	——	——	—
0.1	0.0005	592.0	5.224	0.00000597	0.0000	0.0005	5
0.2	0.0010	540.0	4.345	0.0000452	0.0001	0.0009	9
0.3	0.0015	513.0	3.888	0.000129	0.0001	0.0014	14
0.4	0.0020	497.5	3.627	0.000237	0.0002	0.0018	18
0.5	0.0025	485.5	3.424	0.000378	0.0004	0.0021	21
0.6	0.0030	476.0	3.263	0.000546	0.0006	0.0024	24
0.7	0.0035	470.5	3.169	0.000704	0.0007	0.0028	28
0.8	0.0040	456.0	2.925	0.00119	0.0012	0.0028	28
0.9	0.0045	453.5	2.883	0.00132	0.0013	0.0032	32
1.0	0.0050	447.5	2.781	0.00166	0.0017	0.0033	33
1.1	0.0055	441.0	2.671	0.00213	0.0021	0.0034	34
1.2	0.0060	434.5	2.561	0.00275	0.0028	0.0032	32
1.3	0.0065	429.0	2.468	0.00340	0.0035	0.0030	30
1.4	0.0070	424.5	2.392	0.00406	0.0041	0.0029	29
1.5	0.0075	421.5	2.342	0.00456	0.0046	0.0029	29
1.6	0.0080	419.5	2.308	0.00493	0.0050	0.0030	30
1.8	0.0090	417.0	2.265	0.00543	0.0055	0.0035	35
2.0	0.0100	415.0	2.232	0.00587	0.0059	0.0041	41
2.5	0.0125	410.5	2.155	0.00700	0.0071	0.0054	54
3.0	0.0150	406.5	2.087	0.00817	0.0083	0.0067	67
3.5	0.0175	402.0	2.012	0.00973	0.0098	0.0077	77
4.0	0.0200	396.5	1.918	0.0120	0.0121	0.0079	79
5.0	0.0250	388.0	1.775	0.0168	0.0170	0.0080	80
6.0	0.0300	383.0	1.691	0.0204	0.0206	0.0094	94

TABLE LXXIX

Electrometric Titration of 200 cc. of 1 Per Cent Teozein Plus Varying Amounts of Hydrochloric Acid at 22° C.

cc. HCl N/1	N	E.M.F.	pH	cH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	——	590.0	5.190	0.00000646	——	——	—
0.1	0.0005	504.0	3.736	0.000184	0.0002	0.0003	3
0.2	0.0010	482.0	3.364	0.000433	0.0004	0.0006	6
0.3	0.0015	468.5	3.135	0.000732	0.0008	0.0007	7
0.4	0.0020	461.0	3.009	0.000979	0.0010	0.0010	10
0.5	0.0025	454.5	2.899	0.00126	0.0013	0.0012	12
0.6	0.0030	451.5	2.849	0.00142	0.0014	0.0016	16
0.7	0.0035	446.5	2.763	0.00172	0.0018	0.0017	17
0.8	0.0040	442.5	2.696	0.00201	0.0021	0.0019	19
0.9	0.0045	438.0	2.620	0.00240	0.0024	0.0021	21
1.0	0.0050	435.0	2.570	0.00269	0.0028	0.0022	22
1.1	0.0055	431.5	2.511	0.00309	0.0032	0.0023	23
1.2	0.0060	429.5	2.477	0.00334	0.0035	0.0025	25
1.3	0.0065	427.0	2.434	0.00368	0.0038	0.0027	27
1.4	0.0070	424.5	2.392	0.00406	0.0042	0.0028	28
1.5	0.0075	422.0	2.350	0.00447	0.0046	0.0029	29
1.6	0.0080	420.0	2.316	0.00484	0.0050	0.0030	30
1.8	0.0090	416.5	2.256	0.00554	0.0057	0.0033	33
2.0	0.0100	413.5	2.207	0.00622	0.0064	0.0036	36
2.5	0.0125	407.5	2.105	0.00784	0.0080	0.0045	45
3.0	0.0150	403.0	2.029	0.00936	0.0095	0.0055	55
3.5	0.0175	399.0	1.961	0.0109	0.0110	0.0065	65
4.0	0.0200	395.5	1.902	0.0126	0.0128	0.0072	72
5.0	0.0250	390.0	1.809	0.0155	0.0157	0.0093	93
6.0	0.0300	384.0	1.707	0.0196	0.0198	0.0102	102

TABLE LXXX

Electrometric Titration of 200 cc. of 1 Per Cent Casein Plus Varying Amounts of Hydrochloric Acid at 22° C.

cc. HCl N/1	N	E.M.F.	pH	cH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	——	560.0	4.683	0.0000208	——	——	—
0.1	0.0005	522.0	4.040	0.0000911	0.0001	0.0004	4
0.2	0.0010	503.0	3.719	0.000191	0.0002	0.0008	8
0.3	0.0015	492.0	3.533	0.000294	0.0003	0.0012	12
0.4	0.0020	486.5	3.440	0.000363	0.0004	0.0016	16
0.5	0.0025	482.0	3.364	0.000433	0.0004	0.0021	21
0.6	0.0030	478.5	3.304	0.000496	0.0005	0.0025	25
0.7	0.0035	475.5	3.255	0.000557	0.0006	0.0029	29
0.8	0.0040	469.5	3.153	0.000703	0.0007	0.0033	33
0.9	0.0045	466.5	3.102	0.000791	0.0008	0.0037	37
1.0	0.0050	463.0	3.043	0.000906	0.0010	0.0040	40
1.1	0.0055	459.5	2.984	0.001015	0.0011	0.0044	44
1.2	0.0060	459.5	2.984	0.001015	0.0011	0.0049	49
1.3	0.0065	454.0	2.891	0.00129	0.0013	0.0052	52
1.4	0.0070	452.0	2.857	0.00139	0.0014	0.0056	56
1.5	0.0075	449.5	2.815	0.00153	0.0016	0.0059	59
1.6	0.0080	447.0	2.772	0.00169	0.0018	0.0062	62
1.8	0.0090	442.8	2.688	0.00205	0.0021	0.0069	69
2.0	0.0100	436.8	2.603	0.00249	0.0026	0.0074	74
2.5	0.0125	424.5	2.392	0.00406	0.0042	0.0083	83
3.0	0.0150	416.0	2.249	0.00565	0.0058	0.0092	92
3.5	0.0175	410.5	2.155	0.00700	0.0071	0.0104	104
4.0	0.0200	407.0	2.096	0.00801	0.0082	0.0118	118
5.0	0.0250	400.0	1.978	0.0105	0.0107	0.0143	143
6.0	0.0300	392.0	1.843	0.0143	0.0145	0.0155	155

TABLE LXXXI

Electrometric Titration of 200 cc. of 1 Per Cent Fibrin. Plus Varying Amounts of Hydrochloric Acid at 22° C.

cc. HCl N/1	N	E.M.F.	pH	cH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	——	570.0	4.852	0.0000141	——	——	—
0.1	0.0005	523.5	4.066	0.0000865	0.0001	0.0004	4
0.2	0.0010	502.5	3.710	0.000195	0.0002	0.0008	8
0.3	0.0015	489.5	3.492	0.000321	0.0003	0.0012	12
0.4	0.0020	488.5	3.474	0.000335	0.0004	0.0016	16
0.5	0.0025	487.5	3.458	0.000350	0.0004	0.0021	21
0.6	0.0030	486.5	3.440	0.000363	0.0004	0.0026	26
0.7	0.0035	483.5	3.390	0.000408	0.0004	0.0031	31
0.8	0.0040	479.0	3.310	0.000486	0.0005	0.0035	35
0.9	0.0045	473.5	3.221	0.000603	0.6006	0.0039	39
1.0	0.0050	469.5	3.153	0.000704	0.0007	0.0043	43
1.1	0.0055	465.5	3.086	0.000822	0.0009	0.0046	46
1.2	0.0060	461.5	3.018	0.000961	0.0010	0.0050	50
1.3	0.0065	462.0	3.026	0.000942	0.0010	0.0055	55
1.4	0.0070	461.0	3.009	0.000979	0.0010	0.0060	60
1.5	0.0075	457.0	2.942	0.00114	0.0012	0.0063	63
1.6	0.0080	455.0	2.908	0.00124	0.0013	0.0067	67
1.8	0.0090	449.0	2.806	0.00156	0.0016	0.0074	74
2.0	0.0100	440.0	2.654	0.00222	0.0023	0.0077	77
2.5	0.0125	431.0	2.502	0.00315	0.0032	0.0093	93
3.0	0.0150	423.5	2.376	0.00422	0.0043	0.0107	107
3.5	0.0175	416.0	2.249	0.00565	0.0057	0.0118	118
4.0	0.0200	410.5	2.155	0.00700	0.0072	0.0128	128
5.0	0.0250	403.0	2.029	0.00936	0.0095	0.0155	155
6.0	0.0300	396.0	1.910	0.0123	0.0125	0.0175	175

TABLE LXXXII

ELECTROMETRIC TITRATION OF 200 CC. OF 1 PER CENT DURUMIN PLUS VARYING AMOUNTS OF HYDROCHLORIC ACID AT 35° C.

cc. HCl N/1	N	E.M.F.	pH	cH	N'	n	Gm. equiv. bound per gm. protein $\times 10^{-5}$
—	—	630.0	5.866	0.00000136	—	—	—
0.1	0.0005	575.0	4.936	0.0000116	0.0000	0.0005	5
0.2	0.0010	543.0	4.395	0.0000402	0.0000	0.0010	10
0.3	0.0015	519.0	3.990	0.000102	0.0001	0.0014	14
0.4	0.0020	499.0	3.652	0.000223	0.0002	0.0018	18
0.5	0.0025	488.0	3.466	0.000343	0.0003	0.0022	22
0.6	0.0030	479.0	3.313	0.000486	0.0004	0.0026	26
0.7	0.0035	469.0	3.144	0.000717	0.0007	0.0028	28
0.8	0.0040	459.0	2.975	0.00106	0.0010	0.0030	30
0.9	0.0045	452.5	2.865	0.00137	0.0013	0.0032	32
1.0	0.0050	446.5	2.763	0.00172	0.0017	0.0033	33
1.1	0.0055	441.0	2.671	0.00213	0.0020	0.0035	35
1.2	0.0060	438.0	2.620	0.00240	0.0023	0.0037	37
1.3	0.0065	433.5	2.545	0.00286	0.0028	0.0037	37
1.4	0.0070	429.0	2.468	0.00340	0.0033	0.0037	37
1.5	0.0075	426.5	2.425	0.00375	0.0036	0.0039	39
1.6	0.0080	423.0	2.367	0.00430	0.0042	0.0038	38
1.8	0.0090	420.0	2.316	0.00483	0.0047	0.0043	43
2.0	0.0100	417.5	2.276	0.00538	0.0052	0.0048	48
2.5	0.0125	411.0	2.164	0.00686	0.0067	0.0058	58
3.0	0.0150	406.5	2.087	0.00817	0.0080	0.0070	70
3.5	0.0175	403.5	2.038	0.00918	0.0090	0.0085	85
4.0	0.0200	398.5	1.952	0.0111	0.0108	0.0092	92
5.0	0.0250	391.0	1.829	0.0149	0.0147	0.0103	103
6.0	0.0300	384.0	1.707	0.0197	0.0195	0.0105	105

TABLE LXXXIII

ELECTROMETRIC TITRATION OF 200 CC. OF 1 PER CENT TEOZEIN PLUS VARYING AMOUNTS OF HYDROCHLORIC ACID AT 35° C.

cc. HCl N/1	N	E.M.F.	pH	cH	N'	n	Gm. equiv. bound per gm. protein $\times 10^{-5}$
—	—	590.0	5.190	0.00000646	—	—	—
0.1	0.0005	505.0	3.753	0.000177	0.0001	0.0004	4
0.2	0.0010	483.0	3.381	0.000416	0.0004	0.0006	6
0.3	0.0015	471.0	3.178	0.000663	0.0006	0.0009	9
0.4	0.0020	462.5	3.034	0.000924	0.0009	0.0011	11
0.5	0.0025	455.0	2.908	0.00124	0.0012	0.0013	13
0.6	0.0030	449.5	2.817	0.00153	0.0015	0.0015	15
0.7	0.0035	445.0	2.739	0.00183	0.0018	0.0017	17
0.8	0.0040	440.5	2.662	0.00217	0.0021	0.0019	19
0.9	0.0045	437.0	2.603	0.00249	0.0024	0.0021	21
1.0	0.0050	434.5	2.561	0.00274	0.0027	0.0023	23
1.1	0.0055	432.0	2.519	0.00303	0.0030	0.0025	25
1.2	0.0060	429.5	2.477	0.00334	0.0034	0.0026	26
1.3	0.0065	427.5	2.443	0.00362	0.0035	0.0030	30
1.4	0.0070	425.5	2.410	0.00392	0.0038	0.0032	32
1.5	0.0075	423.0	2.369	0.00430	0.0042	0.0033	33
1.6	0.0080	421.0	2.333	0.00465	0.0046	0.0034	34
1.8	0.0090	417.5	2.274	0.00523	0.0051	0.0039	39
2.0	0.0100	414.0	2.215	0.00610	0.0060	0.0040	40
2.5	0.0125	407.5	2.105	0.00786	0.0078	0.0047	47
3.0	0.0150	403.0	2.029	0.00936	0.0092	0.0058	58
3.5	0.0175	399.5	1.970	0.0107	0.0105	0.0070	70
4.0	0.0200	395.5	1.902	0.0126	0.0124	0.0076	76
5.0	0.0250	389.0	1.792	0.0162	0.0160	0.0090	90
6.0	0.0300	384.0	1.707	0.0197	0.0195	0.0105	105

TABLE LXXXIV

Electrometric Titration of 200 cc. of 1 Per Cent Casein Plus Varying Amounts of Hydrochloric Acid at 35° C.

cc. HCl N/1	N	E.M.F.	pH	cH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	—	560.0	4.683	0.0000208	—	—	—
0.1	0.0005	519.0	3.990	0.000102	0.0001	0.0004	4
0.2	0.0010	499.0	3.652	0.000223	0.0002	0.0008	8
0.3	0.0015	487.5	3.458	0.000350	0.0003	0.0012	12
0.4	0.0020	485.0	3.415	0.000385	0.0004	0.0016	16
0.5	0.0025	480.0	3.330	0.000468	0.0004	0.0021	21
0.6	0.0030	477.0	3.280	0.000525	0.0005	0.0025	25
0.7	0.0035	476.0	3.273	0.000547	0.0005	0.0030	30
0.8	0.0040	475.0	3.246	0.000568	0.0005	0.0035	35
0.9	0.0045	469.5	3.153	0.000703	0.0006	0.0039	39
1.0	0.0050	465.0	3.077	0.000838	0.0008	0.0042	42
1.1	0.0055	463.0	3.043	0.000906	0.0008	0.0047	47
1.2	0.0060	460.5	3.000	0.00100	0.0009	0.0051	51
1.3	0.0065	459.0	2.975	0.00106	0.0010	0.0055	55
1.4	0.0070	456.0	2.925	0.00119	0.0011	0.0059	59
1.5	0.0075	452.5	2.865	0.00137	0.0013	0.0062	62
1.6	0.0080	449.0	2.806	0.00156	0.0014	0.0066	66
1.8	0.0090	445.0	2.739	0.00183	0.0017	0.0073	73
2.0	0.0100	440.0	2.654	0.00221	0.0021	0.0079	79
2.5	0.0125	427.0	2.434	0.00368	0.0035	0.0090	90
3.0	0.0150	419.0	2.299	0.00502	0.0049	0.0101	101
3.5	0.0175	411.0	2.164	0.00686	0.0067	0.0108	108
4.0	0.0200	407.5	2.105	0.00786	0.0077	0.0123	123
5.0	0.0250	399.5	1.970	0.0107	0.0105	0.0145	145
6.0	0.0300	392.0	1.843	0.0143	0.0141	0.0159	159

TABLE LXXXV

Electrometric Titration of 200 cc. of 1 Per Cent Fibrin Plus Varying Amounts of Hydrochloric Acid at 35° C.

cc. HCl N/1	N	E.M.F.	pH	cH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	—	570.0	4.852	0.0000141	—	—	—
0.1	0.0005	540.0	4.345	0.0000452	0.0000	0.0005	5
0.2	0.0010	525.0	4.091	0.0000811	0.0001	0.0009	9
0.3	0.0015	507.0	3.787	0.000163	0.0002	0.0013	13
0.4	0.0020	498.0	3.635	0.000232	0.0002	0.0018	18
0.5	0.0025	490.0	3.500	0.000317	0.0003	0.0022	22
0.6	0.0030	488.5	3.474	0.000335	0.0003	0.0027	27
0.7	0.0035	484.0	3.398	0.000400	0.0004	0.0031	31
0.8	0.0040	480.5	3.338	0.000468	0.0005	0.0035	35
0.9	0.0045	475.0	3.246	0.000568	0.0006	0.0039	39
1.0	0.0050	470.0	3.161	0.000690	0.0007	0.0043	43
1.1	0.0055	466.5	3.102	0.000791	0.0008	0.0047	47
1.2	0.0060	463.0	3.043	0.000906	0.0009	0.0051	51
1.3	0.0065	461.0	3.009	0.000979	0.0010	0.0055	55
1.4	0.0070	459.0	2.975	0.00106	0.0010	0.0060	60
1.5	0.0075	457.0	2.942	0.00114	0.0011	0.0064	64
1.6	0.0080	454.5	2.899	0.00126	0.0012	0.0068	68
1.8	0.0090	450.0	2.823	0.00150	0.0015	0.0075	75
2.0	0.0100	446.0	2.756	0.00176	0.0017	0.0083	83
2.5	0.0125	435.5	2.579	0.00264	0.0026	0.0099	99
3.0	0.0150	426.5	2.425	0.00375	0.0037	0.0113	113
3.5	0.0175	419.0	2.299	0.00502	0.0049	0.0126	126
4.0	0.0200	413.0	2.198	0.00634	0.0063	0.0137	137
5.0	0.0250	404.5	2.053	0.00893	0.0089	0.0161	161
6.0	0.0300	397.0	1.927	0.0118	0.0117	0.0183	183

TABLE LXXXVI

Electrometric Titration of 200 cc. of 1 Per Cent Durumin Plus Varying Amounts of Sodium Hydroxide at 22° C.

cc. NaOH N/1	N	E.M.F.	pH	cOH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	—	630.0	5.866	7.44×10^{-9}	—	—	—
0.1	0.0005	800.0	8.740	5.56×10^{-6}	0.0000	0.0005	5
0.2	0.0010	880.5	10.101	0.000128	0.0002	0.0008	8
0.3	0.0015	897.5	10.389	0.000248	0.0003	0.0012	12
0.4	0.0020	909.0	10.583	0.000388	0.0005	0.0015	15
0.5	0.0025	916.0	10.701	0.000510	0.0007	0.0018	18
0.6	0.0030	923.5	10.829	0.000679	0.0009	0.0021	21
0.7	0.0035	930.0	10.938	0.000875	0.0012	0.0023	23
0.8	0.0040	934.5	11.013	0.001048	0.0015	0.0025	25
0.9	0.0045	939.0	11.090	0.00124	0.0018	0.0027	27
1.0	0.0050	942.0	11.141	0.00140	0.0020	0.0030	30
1.1	0.0055	943.0	11.158	0.00145	0.0020	0.0035	35
1.2	0.0060	944.5	11.182	0.00155	0.0022	0.0038	38
1.3	0.0065	945.0	11.191	0.00157	0.0022	0.0043	43
1.4	0.0070	946.5	11.216	0.00167	0.0023	0.0047	47
1.5	0.0075	949.0	11.259	0.00184	0.0026	0.0049	49
1.6	0.0080	951.0	11.293	0.00199	0.0028	0.0052	52
1.8	0.0090	954.0	11.344	0.00223	0.0031	0.0059	59
2.0	0.0100	956.5	11.385	0.00246	0.0034	0.0066	66
2.5	0.0125	963.0	11.496	0.00317	0.0045	0.0080	80
3.0	0.0150	968.0	11.580	0.00385	0.0054	0.0096	96
3.5	0.0175	970.0	11.614	0.00416	0.0058	0.0117	117
4.0	0.0200	973.0	11.665	0.00469	0.0066	0.0134	134
5.0	0.0250	979.5	11.775	0.00601	0.0085	0.0165	165
6.0	0.0300	987.5	11.910	0.00821	0.0116	0.0184	184

TABLE LXXXVII

Electrometric Titration of 200 cc. of 1 Per Cent Teozein Plus Varying Amounts of Sodium Hydroxide at 22° C.

cc. NaOH N/1	N	E.M.F.	pH	cOH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	—	590.0	5.190	1.57×10^{-9}	—	—	—
0.1	0.0005	807.5	8.868	0.00000744	0.0000	0.0005	5
0.2	0.0010	860.0	9.755	0.0000575	0.0001	0.0009	9
0.3	0.0015	895.0	10.346	0.000224	0.0003	0.0012	12
0.4	0.0020	904.5	10.506	0.000326	0.0004	0.0016	16
0.5	0.0025	915.5	10.693	0.000500	0.0007	0.0018	18
0.6	0.0030	920.0	10.769	0.000594	0.0008	0.0022	22
0.7	0.0035	928.0	10.904	0.000810	0.0011	0.0024	24
0.8	0.0040	934.5	11.013	0.001059	0.0014	0.0026	26
0.9	0.0045	938.5	11.081	0.00122	0.0017	0.0028	28
1.0	0.0050	942.0	11.111	0.00140	0.0020	0.0030	30
1.1	0.0055	945.5	11.200	0.00160	0.0022	0.0033	33
1.2	0.0060	948.0	11.242	0.00177	0.0025	0.0035	35
1.3	0.0065	950.0	11.276	0.00191	0.0027	0.0038	38
1.4	0.0070	952.0	11.310	0.00207	0.0028	0.0042	42
1.5	0.0075	954.0	11.344	0.00224	0.0030	0.0045	45
1.6	0.0080	955.5	11.370	0.00237	0.0032	0.0048	48
1.8	0.0090	958.5	11.419	0.00266	0.0037	0.0053	53
2.0	0.0100	961.5	11.471	0.00299	0.0042	0.0058	58
2.5	0.0125	968.5	11.588	0.00393	0.0055	0.0070	70
3.0	0.0150	973.5	11.674	0.00478	0.0065	0.0085	85
3.5	0.0175	978.0	11.749	0.00569	0.0080	0.0095	95
4.0	0.0200	982.5	11.825	0.00676	0.0095	0.0105	105
5.0	0.0250	989.0	11.935	0.00872	0.0120	0.0130	130
6.0	0.0300	994.5	12.028	0.0108	0.0151	0.0149	149

TABLE LXXXVIII

Electrometric Titration of 200 cc. of 1 Per Cent Casein Plus Varying Amounts of Sodium Hydroxide at 22° C.

cc. NaOH N/1	N	E.M.F.	pH	cOH	N′	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	——	560.0	4.683	4.87×10^{-10}	——	——	—
0.1	0.0005	613.5	5.588	3.90×10^{-9}	0.0000	0.0005	5
0.2	0.0010	643.0	6.086	1.23×10^{-8}	0.0000	0.0010	10
0.3	0.0015	657.5	6.332	2.17	0.0000	0.0015	15
0.4	0.0020	662.0	6.407	2.58	0.0000	0.0020	20
0.5	0.0025	667.0	6.492	3.14	0.0000	0.0025	25
0.6	0.0030	670.0	6.543	3.53	0.0000	0.0030	30
0.7	0.0035	673.5	6.602	4.05	0.0000	0.0035	35
0.8	0.0040	677.5	6.670	4.73	0.0000	0.0040	40
0.9	0.0045	682.5	6.754	5.74	0.0000	0.0045	45
1.0	0.0050	690.0	6.881	7.67	0.0000	0.0050	50
1.1	0.0055	701.0	7.067	1.18×10^{-7}	0.0000	0.0055	55
1.2	0.0060	715.0	7.303	2.04	0.0000	0.0060	60
1.3	0.0065	728.0	7.523	3.37	0.0000	0.0065	65
1.4	0.0070	739.0	7.709	5.19	0.0000	0.0070	70
1.5	0.0075	761.5	8.090	1.24×10^{-6}	0.0000	0.0075	75
1.6	0.0080	790.0	8.571	3.76	0.0000	0.0080	80
1.8	0.0090	827.5	9.206	0.0000163	0.0000	0.0090	90
2.0	0.0100	857.5	9.713	0.0000522	0.0001	0.0099	99
2.5	0.0125	890.0	10.262	0.000185	0.0002	0.0123	123
3.0	0.0150	909.5	10.592	0.000395	0.0005	0.0145	145
3.5	0.0175	926.5	10.878	0.000765	0.0010	0.0165	165
4.0	0.0200	940.0	11.107	0.00129	0.0018	0.0182	182
5.0	0.0250	958.5	11.419	0.00266	0.0037	0.0213	213
6.0	0.0300	973.5	11.674	0.00478	0.0066	0.0234	234

TABLE LXXXIX

Electrometric Titration of 200 cc. of 1 Per Cent Fibrin Plus Varying Amounts of Sodium Hydroxide at 22° C.

cc. NaOH N/1	N	E.M.F.	pH	cOH	N′	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	——	570.0	4.852	7.18×10^{-10}	——	——	—
0.1	0.0005	601.0	5.376	2.40×10^{-9}	0.0000	0.0005	5
0.2	0.0010	630.0	5.866	7.44	0.0000	0.0010	10
0.3	0.0015	633.0	5.917	8.36	0.0000	0.0015	15
0.4	0.0020	646.5	6.146	1.42×10^{-8}	0.0000	0.0020	20
0.5	0.0025	679.5	6.704	5.11	0.0000	0.0025	25
0.6	0.0030	732.5	7.600	4.02×10^{-7}	0.0000	0.0030	30
0.7	0.0035	785.5	8.496	3.16×10^{-6}	0.0000	0.0035	35
0.8	0.0040	817.0	9.028	0.0000108	0.0000	0.0040	40
0.9	0.0045	846.0	9.518	0.0000333	0.0000	0.0045	45
1.0	0.0050	859.0	9.738	0.0000553	0.0001	0.0049	49
1.1	0.0055	868.5	9.899	0.0000800	0.0001	0.0054	54
1.2	0.0060	875.0	10.008	0.000103	0.0001	0.0059	59
1.3	0.0065	882.5	10.134	0.000138	0.0002	0.0063	63
1.4	0.0070	888.0	10.228	0.000171	0.0002	0.0068	68
1.5	0.0075	892.5	10.304	0.000204	0.0003	0.0072	72
1.6	0.0080	896.0	10.363	0.000234	0.0003	0.0077	77
1.8	0.0090	903.5	10.490	0.000313	0.0004	0.0086	86
2.0	0.0100	910.0	10.600	0.000403	0.0005	0.0095	95
2.5	0.0125	925.0	10.853	0.000723	0.0010	0.0115	115
3.0	0.0150	939.0	11.090	0.00124	0.0017	0.0134	134
3.5	0.0175	947.0	11.225	0.00170	0.0024	0.0151	151
4.0	0.0200	953.0	11.327	0.00215	0.0030	0.0170	170
5.0	0.0250	962.5	11.487	0.00308	0.0041	0.0209	209
6.0	0.0300	972.0	11.648	0.00450	0.0063	0.0237	237

TABLE XC

Electrometric Titration of 200 cc. of 1 Per Cent Durumin Plus Varying Amounts of Sodium Hydroxide at 35° C.

cc. NaOH N/1	N	E.M.F.	pH	cOH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-6}$
—	—	630.0	5.866	7.44×10^{-9}	—	—	—
0.1	0.0005	803.0	8.791	6.25×10^{-9}	0.0000	0.0005	5
0.2	0.0010	879.0	10.076	0.000120	0.0002	0.0008	8
0.3	0.0015	909.5	10.592	0.000395	0.0005	0.0010	10
0.4	0.0020	915.0	10.684	0.000489	0.0006	0.0014	14
0.5	0.0025	919.0	10.752	0.000572	0.0007	0.0018	18
0.6	0.0030	923.0	10.820	0.000666	0.0008	0.0022	22
0.7	0.0035	928.5	10.912	0.000827	0.0010	0.0025	25
0.8	0.0040	933.0	10.989	0.000983	0.0012	0.0028	28
0.9	0.0045	937.5	11.065	0.00117	0.0015	0.0030	30
1.0	0.0050	941.0	11.124	0.00135	0.0017	0.0033	33
1.1	0.0055	942.5	11.150	0.00143	0.0018	0.0037	37
1.2	0.0060	943.0	11.158	0.00145	0.0018	0.0042	42
1.3	0.0065	944.5	11.182	0.00154	0.0019	0.0046	46
1.4	0.0070	945.5	11.200	0.00160	0.0020	0.0050	50
1.5	0.0075	946.0	11.208	0.00164	0.0021	0.0054	54
1.6	0.0080	946.5	11.217	0.00167	0.0021	0.0059	59
1.8	0.0090	951.5	11.302	0.00203	0.0025	0.0065	65
2.0	0.0100	954.5	11.352	0.00228	0.0028	0.0072	72
2.5	0.0125	958.0	11.411	0.00261	0.0032	0.0093	93
3.0	0.0150	963.0	11.496	0.00317	0.0039	0.0111	111
3.5	0.0175	969.0	11.597	0.00400	0.0050	0.0125	125
4.0	0.0200	974.0	11.682	0.00487	0.0061	0.0139	139
5.0	0.0250	981.0	11.800	0.00636	0.0079	0.0171	171
6.0	0.0300	989.0	11.935	0.00872	0.0109	0.0191	191

TABLE XCI

Electrometric Titration of 200 cc. of 1 Per Cent Teozein Plus Varying Amounts of Sodium Hydroxide at 35° C.

cc. NaOH N/1	N	E.M.F.	pH	cOH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-6}$
—	—	590.0	5.190	1.57×10^{-9}	—	—	—
0.1	0.0005	820.0	9.078	0.0000121	0.0000	0.0005	5
0.2	0.0010	870.0	9.924	0.0000850	0.0001	0.0009	9
0.3	0.0015	896.0	10.363	0.000234	0.0003	0.0012	12
0.4	0.0020	905.5	10.524	0.000341	0.0004	0.0016	16
0.5	0.0025	915.5	10.693	0.000500	0.0006	0.0019	19
0.6	0.0030	922.0	10.803	0.000642	0.0008	0.0022	22
0.7	0.0035	927.5	10.896	0.000793	0.0010	0.0025	25
0.8	0.0040	932.0	10.972	0.000948	0.0012	0.0028	28
0.9	0.0045	936.5	11.047	0.00113	0.0014	0.0031	31
1.0	0.0050	941.0	11.124	0.00135	0.0017	0.0033	33
1.1	0.0055	944.0	11.175	0.00151	0.0019	0.0036	36
1.2	0.0060	946.5	11.216	0.00166	0.0021	0.0039	39
1.3	0.0065	948.5	11.250	0.00181	0.0023	0.0042	42
1.4	0.0070	950.5	11.284	0.00195	0.0025	0.0045	45
1.5	0.0075	953.0	11.327	0.00215	0.0027	0.0048	48
1.6	0.0080	955.0	11.361	0.00232	0.0029	0.0051	51
1.8	0.0090	948.0	11.411	0.00261	0.0032	0.0058	58
2.0	0.0100	961.0	11.462	0.00293	0.0036	0.0064	64
2.5	0.0125	969.5	11.606	0.00408	0.0051	0.0074	74
3.0	0.0150	975.0	11.699	0.00506	0.0063	0.0087	87
3.5	0.0175	979.5	11.775	0.00603	0.0075	0.0100	100
4.0	0.0200	985.0	11.868	0.00744	0.0093	0.0107	107
5.0	0.0250	989.0	11.935	0.00872	0.0109	0.0141	141
6.0	0.0300	995.0	12.037	0.0110	0.0137	0.0163	163

TABLE XCII

ELECTROMETRIC TITRATION OF 200 CC. OF 1 PER CENT CASEIN PLUS VARYING AMOUNTS OF SODIUM HYDROXIDE AT 35° C.

cc. NaOH N/1	N	E.M.F.	pH	cOH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	—	560.0	4.683	4.87×10^{-10}	—	—	—
0.1	0.0005	632.0	5.900	8.03×10^{-9}	0.0000	0.0005	5
0.2	0.0010	647.0	6.154	1.44×10^{-8}	0.0000	0.0010	10
0.3	0.0015	658.5	6.349	2.25	0.0000	0.0015	15
0.4	0.0020	663.0	6.424	2.68	0.0000	0.0020	20
0.5	0.0025	667.0	6.492	3.14	0.0000	0.0025	25
0.6	0.0030	671.0	6.559	3.67	0.0000	0.0030	30
0.7	0.0035	681.0	6.728	5.41	0.0000	0.0035	35
0.8	0.0040	689.0	6.864	7.39	0.0000	0.0040	40
0.9	0.0045	693.5	6.940	8.80	0.0000	0.0045	45
1.0	0.0050	697.0	6.999	1.01×10^{-7}	0.0000	0.0050	50
1.1	0.0055	707.0	7.168	1.49	0.0000	0.0055	55
1.2	0.0060	716.5	7.329	2.16	0.0000	0.0060	60
1.3	0.0065	729.0	7.540	3.51	0.0000	0.0065	65
1.4	0.0070	740.0	7.726	5.38	0.0000	0.0070	70
1.5	0.0075	763.0	8.115	1.32×10^{-6}	0.0000	0.0075	75
1.6	0.0080	791.5	8.597	4.00	0.0000	0.0080	80
1.8	0.0090	827.0	9.197	0.0000159	0.0000	0.0090	90
2.0	0.0100	846.0	9.518	0.0000333	0.0001	0.0099	99
2.5	0.0125	879.0	10.076	0.000120	0.0002	0.0123	123
3.0	0.0150	900.0	10.431	0.000272	0.0003	0.0147	147
3.5	0.0175	923.0	10.820	0.000666	0.0008	0.0167	167
4.0	0.0200	940.0	11.107	0.00130	0.0016	0.0184	184
5.0	0.0250	959.0	11.428	0.00271	0.0033	0.0217	217
6.0	0.0300	971.0	11.631	0.00432	0.0054	0.0246	246

TABLE XCIII

ELECTROMETRIC TITRATION OF 200 CC. OF 1 PER CENT FIBRIN PLUS VARYING AMOUNTS OF SODIUM HYDROXIDE AT 35° C.

cc. NaOH N/1	N	E. M. F.	pH	cOH	N'	n	Gm. equiv. bound per gm. protein
							$\times 10^{-5}$
—	—	570.0	4.852	7.18×10^{-10}	—	—	—
0.1	0.0005	600.5	5.368	2.36×10^{-9}	0.0000	0.0005	5
0.2	0.0010	631.0	5.883	7.73	0.0000	0.0010	10
0.3	0.0015	636.0	5.968	9.37	0.0000	0.0015	15
0.4	0.0020	645.5	6.129	1.36×10^{-8}	0.0000	0.0020	20
0.5	0.0025	676.0	6.644	4.46	0.0000	0.0025	25
0.6	0.0030	731.5	7.583	3.86×10^{-7}	0.0000	0.0030	30
0.7	0.0035	787.0	8.521	0.00000335	0.0000	0.0035	35
0.8	0.0040	819.0	9.062	0.0000117	0.0000	0.0040	40
0.9	0.0045	847.5	9.544	0.0000354	0.0000	0.0045	45
1.0	0.0050	861.0	9.772	0.0000599	0.0001	0.0049	49
1.1	0.0055	869.0	9.907	0.0000816	0.0001	0.0054	54
1.2	0.0060	879.0	10.076	0.000120	0.0002	0.0058	58
1.3	0.0065	884.0	10.160	0.000146	0.0002	0.0063	63
1.4	0.0070	889.5	10.254	0.000181	0.0002	0.0068	68
1.5	0.0075	893.0	10.313	0.000208	0.0003	0.0072	72
1.6	0.0080	897.5	10.389	0.000248	0.0003	0.0077	77
1.8	0.0090	905.5	10.524	0.000339	0.0004	0.0086	86
2.0	0.0100	914.0	10.668	0.000470	0.0006	0.0094	94
2.5	0.0125	928.5	10.912	0.000827	0.0010	0.0115	115
3.0	0.0150	939.0	11.090	0.00124	0.0015	0.0135	135
3.5	0.0175	950.5	11.284	0.00195	0.0024	0.0151	151
4.0	0.0200	955.5	11.370	0.00237	0.0029	0.0171	171
5.0	0.0250	967.5	11.572	0.00378	0.0047	0.0203	203
6.0	0.0300	978.0	11.749	0.00569	0.0071	0.0229	229

The Relative Amount of Acid or Alkali Bound by the Various Proteins as Determined by the Conductivity Method. These determinations were made by adding different amounts of protein to the same amount of acid or alkali. The conventional Wheatstone bridge setup was employed in measuring the resistances of the various solutions which were placed in a Freas electrical conductivity cell. These resistances, expressed in terms of specific conductivity, are shown in Tables XCIV and XCV. The values represent the actual specific conductivity of the protein-acid or -alkali salt plus that of the free acid or alkali as the specific conductivity caused by the protein or the soluble ash of the protein has been subtracted.

Although the results are not as striking as those obtained by potentiometric methods, they show the same similarity. The proteins prepared from cereals belonging to the same group show similar results. Due to the sources of error as pointed out in the previous section no issue will be made of these experiments except in that they support the results of the potentiometric measurements, showing, by an altogether different method, that *the ions of the acid or alkali are taken out of solution by the protein.*

IV. DISCUSSION

A. Preparation and Chemical Analyses

Preparation of Proteins. In the preparation of the proteins very little difference was noted between the various prolamines of an individual group. For instance, the prolamines from the members of the wheat group appeared almost identical with one another in physical properties at all of the stages of preparation. As compared with the maize group [14] a distinctly different behavior was noted but all of the prolamines of this group were similar. The essential difference between these groups was their insolubility in the more dilute concentrations of alcohol. The prolamines from kafir and sorghum were almost insoluble in cold, 80 per cent alcohol. When the alcoholic solution of the prolamines of this group was poured into water, the proteins separated in a tough, plastic mass while those of the wheat group separated in soft, sticky masses. Secalin, sativin and hordein behave very similar to those of the wheat group.

[14] We have included maize, teosinte, kafir, and sorghum in a corn "group." This grouping may be questioned by some botanists. This grouping was taken up with G. M. Collins of the Bureau of Plant Industry, U. S. Dept. of Agriculture, who replied as follows: "Regarding the relationship of teosinte I think you would be entirely justified in placing teosinte and corn in a group with the sorghums and contrasting this group with the wheats. It is true that corn and teosinte are placed in a separate tribe, Tripsaceæ, but our genetic work has led us to consider the distinction as artificial. Practically the only character that distinguishes the Tripsaceæ from the Andropogoneæ is the segregation of the sexes into separate flowers. In corn and corn x teosinte this distinction breaks down not infrequently. In fact, Hitchcock, in his Genera of Grasses of the United States, says that the Tripsaceæ should be considered as scarcely more than a subtribe of the Andropogoneæ."

TABLE XCIV

Specific Conductivity of 25 cc. of N/50 Hydrochloric Acid Plus Varying Amounts of Protein, at 27° C.*

Protein	0.5000 gram †	0.1000 gram	0.2500 gram	0.4000 gram	0.6000 gram	0.8000 gram	1.0000 gram
Gliadin	0.0001740	0.008196	0.007459	0.006799	0.005752	0.005208	0.004339
Speltin	0.0001080	0.008249	0.007909	0.007647	0.006452	0.005244	0.004232
Durumin	0.0000993	0.007818	0.007188	0.006404	0.005898	0.005164	0.004031
Dicoccumin	0.0001247	0.008051	0.007452	0.006545	0.005916	0.005123	0.004574
Monococcumin	0.0000746	0.007981	0.007298	0.006522	0.005895	0.005286	0.004741
Secalin	0.0000410	0.008036	0.007760	0.006886	0.005992	0.005415	0.004713
Sativin	0.0000954	0.008089	0.007699	0.007378	0.006902	0.006122	0.005515
Hordein	0.0000930	0.007914	0.007950	0.007396	0.007112	0.006698	0.006127
Zein	0.0000880	0.008309	0.008146	0.008119	0.006925	0.006228	0.005530
Teozein	0.0000800	0.007964	0.007893	0.007421	0.006710	0.006071	0.005442
Kafirin	0.0000865	0.008010	0.008033	0.007668	0.007149	0.006681	0.006220
Sorghumin	0.0000536	0.008249	0.008094	0.007841	0.007654	0.007277	0.006951
Casein	0.0000740	0.007223	0.005610	0.004049	0.002590	0.001781	0.001411
Fibrin	0.0001109	0.006852	0.004894	0.003108	0.001949	0.001525	0.001303

* Specific conductivity of N/50 hydrochloric acid, at 27° C. is 0.008662.
† In 25 cc. of conductivity water.

TABLE XCV

SPECIFIC CONDUCTIVITY OF 25 CC. OF N/50 SODIUM HYDROXIDE PLUS VARYING AMOUNTS OF PROTEIN, AT 27° C.*

Protein	0.5000 gram †	0.2500 gram	0.5000 gram	0.7500 gram	1.0000 gram	1.2500 grams	1.5000 grams	1.0000 gram ‡
Gliadin	0.0001740	0.003847	0.003382	0.002836	0.002202	0.001868	0.001537	0.0001680
Speltin	0.0001080	0.004318	0.003789	0.003235	0.002467	0.002140	0.001663	0.0002758
Durumin	0.0000993	0.004201	0.003538	0.003082	0.002820	0.002012	0.002307	0.0003715
Dicoccumin	0.0001247	0.004363	0.003747	0.003560	0.003068	0.002555	0.001958	0.0003286
Monococcumin	0.0000746	0.004085	0.003309	0.002525	0.002238	0.002033	0.001701	0.0002380
Secalin	0.0000410	0.004207	0.003169	0.002724	0.002346	0.002070	0.001746	0.0001800
Sativin	0.0000954	0.003582	0.002709	0.001946	0.001485	0.001118	0.000931	0.0001503
Hordein	0.0000930	0.003614	0.002781	0.002261	0.001749	0.001414	0.001202	0.0001498
Zein	0.0000880	0.003449	0.002724	0.002089	0.001756	0.001429	0.001250	0.0001660
Teozein	0.0000800	0.003627	0.002741	0.002223	0.001786	0.001456	0.001239	0.0001810
Kafirin	0.0000865	0.004025	0.003572	0.003091	0.002621	0.002307	0.001935	0.0002425
Sorghumin	0.0000536	0.002839	0.001709	0.001230	0.001051	0.000941	0.000852	0.0001523
Casein	0.0000740	0.001549	0.001011	0.001000	0.000981	0.000886	0.000905	0.0001760
Fibrin	0.0001109	0.002537	0.001409	0.001199	0.001113	0.001091	0.001063	0.0003231

* Specific conductivity of N/50 sodium hydroxide at 27° C. is 0.004803.
† In 25 cc. of conductivity water.
‡ In 25 cc. of N/250 sodium hydroxide.

Elementary Analysis. An examination of the results of the elementary analysis of the various proteins, Table XCVI, shows that all of the proteins used have a similar elementary composition. There are, however, several rather marked differences which divide the prolamines into two distinct classes or groups. The maize type, zein, teozein, kafirin and sorghumin average somewhat higher in carbon and hydrogen and have a lower nitrogen and sulfur content than do the proteins from the wheat group. The rather high carbon content of durumin, monococcumin and dicoccumin as compared to those of gliadin and speltin are noteworthy but the evidence afforded is not sufficient to draw any conclusions concerning a possible difference in their chemical composition. The nitrogen content of sorghumin is much lower than in any of the other prolamines thus far examined. This may be due to a difference in the chemical composition of the protein. Visco (1921) also reports a low nitrogen content (13.61%) for the alcohol soluble protein of sorghum. As stated in the previous section, all of the color could not be removed from the prolamine from sorghum by extracting with absolute alcohol and with ether.

Nitrogen Distribution. The nitrogen distribution of the various proteins as determined by the Van Slyke analysis and expressed in per cent of total nitrogen as given in Table XCVII, again shows a close relationship between the proteins of the same cereal group but differences between the proteins from the different groups. The percentages of ammonia nitrogen for the wheat series is about 24.50 per cent as compared to 19.00 per cent for the maize group. These values are much larger than for those of casein and fibrin which have an ammonia nitrogen content of approximately 10.00 per cent and 7.00 per cent respectively.

Under humin nitrogen, no consistent difference is noted. As the amount of humin formed is in general more dependent upon the second factor necessary for humin formation (probably an aldehyde) than upon either tryptophane or tyrosine content, the humin figures give no information as to the content of these two amino acids.

Considerable difference was noted between the various proteins in the percentages of basic nitrogen. The prolamines of the wheat group have approximately the same amount of basic nitrogen. This also holds for the individual basic amino acids. Hordein differs in that it has an exceptionally high percentage of histidine nitrogen. This may be due in part to some of the proline precipitating in this fraction, a possibility that has been pointed out by Sandstrom (1924). This postulation is substantiated by the low non-amino nitrogen fraction in the filtrate from the bases. A study of the proline content of hordein as recorded in the literature shows that more of this amino acid has been obtained from hordein than from any of the other prolamines analyzed. The basic nitrogen of the maize group is much lower than in any of the

TABLE XCVI

ELEMENTARY ANALYSIS OF THE VARIOUS PROTEINS

Protein	Carbon	Hydrogen	Nitrogen	Sulfur	Oxygen	Author
	per cent	per cent	per cent	per cent	per cent	
Gliadin	52.72	6.86	17.66	1.14	21.62	Osborne and Harris (1906).
Speltin	52.31	7.04	17.02	1.20	22.43	This paper.
Durumin	54.27	6.61	17.53	0.99	20.60	This paper.
Dicoccumin	55.50	6.89	17.56	1.20	18.85	This paper.
Monococcumin	55.74	6.88	16.68	1.14	19.56	This paper.
Secalin	52.75	6.84	17.72	1.21	21.48	Osborne (1895).
Sativin	53.06	6.94	16.38	2.26	21.36	Osborne (1892).
Hordein	54.29	6.80	17.21	0.83	20.87	Osborne (1895a).
Zein	55.23	7.26	16.13	0.60	20.78	Chittenden and Osborne (1892).
Teozein	57.67	7.42	15.91	0.59	18.41	This paper.
Kafirin	55.19	7.36	16.44	0.60	20.41	Johns and Brewster (1916).
Sorghumin	55.33	6.89	14.34	0.61	22.83	This paper.
Casein	53.50	7.13	15.80	0.72	22.14 *	Van Slyke and Bosworth (1913).
Fibrin	52.68	6.83	16.91	1.10	22.48	Hammarstein (1880).

* Phosphorus = 0.71%.

TABLE XCVII

THE NITROGEN DISTRIBUTION OF THE VARIOUS PROTEINS AS DETERMINED BY THE VAN SLYKE ANALYSIS, EXPRESSED IN PER CENT OF TOTAL NITROGEN

Protein	Ammonia N	Humin N		Basic N				N in filtrate from bases		Total N	Total Basic N
		insoluble	soluble	arginine	histidine	cystine	lysine	amino	non-amino		
Gliadin	24.61	0.52	0.35	6.38	5.41	1.68	0.57	53.49	6.14	99.15	14.04
Speltin	24.18	0.49	0.56	6.58	4.45	1.02	3.07	53.49	5.50	99.34	15.12
Durumin	25.34	0.38	0.46	6.44	3.88	1.33	1.88	53.98	6.53	100.22	13.53
Dicoccumin	23.89	0.58	0.35	7.99	2.65	1.57	2.95	53.65	6.51	100.14	15.16
Monococcumin	24.19	0.89	0.64	7.20	5.05	1.13	2.30	53.05	4.50	99.05	15.68
Secalin	22.18	0.80	0.37	6.80	7.21	1.44	0.42	50.14	9.77	99.13	15.87
Sativin	22.20	0.70	0.55	8.93	2.31	1.74	1.20	54.60	5.86	98.08	14.18
Hordein	23.38	1.04	0.40	6.22	10.36	1.38	3.02	50.41	3.65	100.26	20.98
Zein	18.06	0.61	0.51	3.92	2.45	0.98	0.89	66.08	5.90	99.40	8.24
Teozein	18.99	0.69	0.50	3.90	3.58	0.80	2.57	64.44	3.62	99.09	10.85
Kafirin	20.76	0.69	0.66	3.92	1.71	1.23	2.48	68.85	0.32	100.62	9.34
Sorghumin	18.96	2.29	0.79	4.83	1.19	0.92	3.45	60.01	5.33	97.77	10.39
Casein	10.20	0.34	1.17	9.20	6.26	1.05	8.49	54.12	8.76	99.59	25.00
Fibrin	6.93	1.44	1.48	14.16	4.30	0.89	14.06	55.86	0.23	99.35	33.41

other prolamines and is probably lower than in any other group of proteins. Casein and fibrin both have a very high basic nitrogen content. This series of prolamines have a basic nitrogen content ranging from 8 per cent to 20 per cent. The lysine content of all of the prolamines is very low while the cystine content is nearly uniform for all of the proteins, averaging about one per cent of the total nitrogen.

The nitrogen in the filtrate from the bases shows the same variations as do the basic nitrogen figures. In the prolamines from the wheat series the nitrogen in the filtrate from the bases is about 60 per cent of the total, while in the case of those from the maize type of cereals, it is about 70 per cent.

Leaving the protamines out of consideration, this series of proteins offer about as wide a variation in the nitrogen distribution as could be found in any equal number of proteins. The ammonia nitrogen fraction varies from 6.93 per cent in fibrin to 25.34 per cent in durumin, the total basic nitrogen fraction varies from 8 per cent in zein to 33 per cent in fibrin, the arginine fraction varies from 3.9 per cent in teozein to 14.16 per cent in fibrin and the lysine fraction varies from 0.42 per cent in secalin to 14.06 per cent in fibrin. These variations, with those found in the dicarboxylic acids, make this set of proteins very desirable for studying acid and alkali binding capacities in relation to chemical composition.

Tryptophane and Cystine Content. The content of the amino acids tryptophane and cystine in the various proteins are shown in table XCVIII. These were determined by Dr. D. Breese Jones, of the Bureau

TABLE XCVIII

THE TRYPTOPHANE AND CYSTINE CONTENT OF THE VARIOUS PROTEINS [18]

	Our preparations		Bureau of Chemistry preparations	
	Tryptophane	Cystine	Tryptophane	Cystine
	Per cent	Per cent	Per cent	Per cent
Gliadin	0.71	1.68	1.09	1.76
Speltin	1.08	1.79		
Durumin	1.09	1.42		
Dicoccumin	0.80	1.98		
Monococcumin	0.48	1.85		
Secalin	0.36	2.64		
Sativin	none	3.48		
Hordein	0.45	1.47	1.06	1.55
Zein	none	0.81		
Teozein	none	1.02		
Kafirin	0.73	0.53	1.17	0.59
Sorghumin	none	0.86		
Casein	2.20	0.27	2.22	0.25
Fibrin	4.40	3.72		

[18] These determinations were kindly made by Dr. D. Breese Jones of the Bureau of Chemistry, U. S. D. A. We are indebted to Dr. Jones for permission to use these data in advance of his own publication.

of Chemistry. More striking differences between the proteins prepared from cereals of the same group are shown here than are shown by any of the other analyses. This is especially noted in the case of the maize group. Zein, teozein and sorghumin do not contain tryptophane while kafirin does. Kafirin has a much lower cystine content than do the other members of this group. Sativin is also peculiar in that it

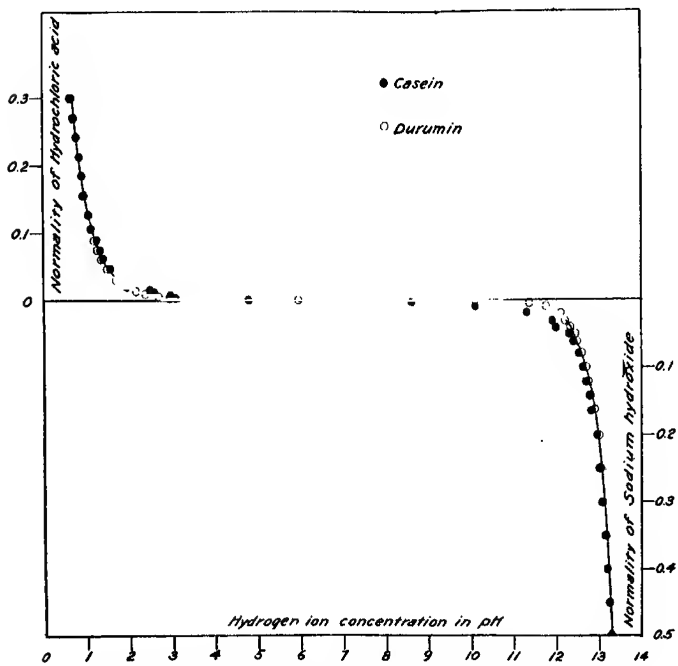


FIG. 3.—Buffer curves of durumin and casein in various normalities of hydrochloric acid and sodium hydroxide.

does not contain tryptophane, and that it has an exceptionally high cystine content. There is also a striking difference between the cystine and tryptophane content of secalin and gliadin, the prolamines which have been considered as identical by some investigators.

General Considerations. When considering the above results as well as the color tests, Table XV, the true ammonia nitrogen, Table XVI, the free amino nitrogen of the native proteins, Table XVII and the free carboxyl groups of the native proteins, Table XVIII, no marked differences are noted between the proteins of the different

cereals of the same group. Gliadin, speltin, durumin, dicoccumin and monococcumin all have approximately the same analyses. Although there are differences, no clean-cut division can be made between the members of one group. Secalin can also be included in the wheat group. Sativin and hordein are intermediate between the wheat and maize groups. Zein, teozein, kafirin and sorghumin all show similar analyses. Casein and fibrin both have a different chemical composition but this is to be expected as they both belong to different classes of proteins.

B. Physico-Chemical Properties

Hydrochloric Acid and Sodium Hydroxide Binding Measured Potentiometrically. The first attempt to compare the results of the titration curves was made by plotting the buffer curves as shown in Fig. 3 where pH is taken as the abscissa and the normality of the original acid and alkali concentrations as the ordinate. Curves for only two proteins, durumin and casein were plotted. The line is drawn for the points given by durumin. The curves of the remaining proteins would lie so close to the plotted curves that it would be impossible to distinguish between them had all been included. The curves consist of a flat portion where a very small increase in the concentration of acid or alkali causes a large change in pH. When, however, the acid concentration is greater than pH 2.5 or the alkali concentration is greater than pH 10.5 the proteins become good buffers and the increases in the acid or alkali concentration cause relatively little change in pH.

Almost all of the points fall exactly on a smooth curve. This is the type of curve that Loeb and coworkers used in most of their work. Due to the fact that a logarithmic function is plotted against the actual values, a much smoother curve is obtained than when the actual numbers are used in both cases.

In examining the results of the acid and alkali binding capacities of the various proteins, it is readily seen that no one point can be taken for comparison as the experimental error is too large. If, however, curves are drawn as shown in Fig. 4 for acid and Fig. 5 for alkali, comparisons can be made from the average curves. Only the curves for durumin and casein are given as representative of the series. The curves are very similar especially at the higher concentrations of acid and alkali. The amount of acid or alkali bound at the higher concentrations is approximately the same for every protein regardless of the amount of amide nitrogen, basic nitrogen or any other difference in the chemical constitution. However, there is a difference at the lower concentrations of acid and alkali where the proteins with the higher basic nitrogen content bind more acid and alkali.

These results, as do those of most other workers, show a smooth,

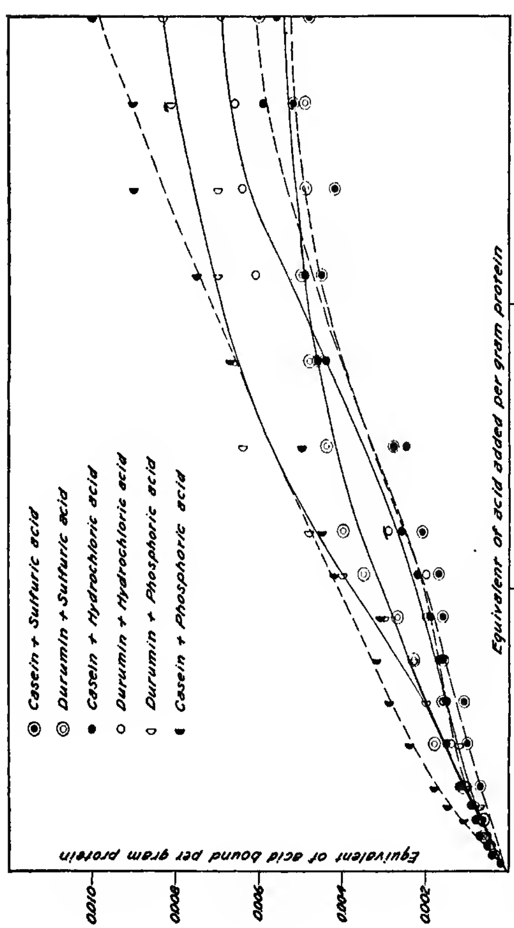


FIG. 4.—The relation of acid added to acid bound by durumin and casein for hydrochloric, sulfuric and phosphoric acid.

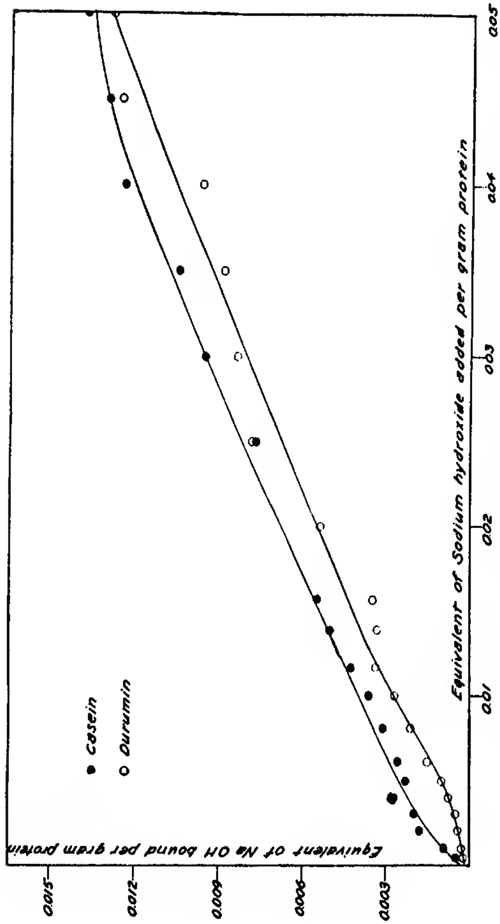


FIG. 5.—The relation of sodium hydroxide added to sodium hydroxide bound by durumin and casein.

regular curve rather than a broken one as reported by Lloyd and Mays (1922) for gelatin and hydrochloric acid. There appears to be no point where the protein is "saturated" with acid or alkali, that is, fails to bind more when the original concentration is increased. In all cases where the temperature was approximately 22° C. there was a progres-

TABLE XCIX

CALCULATION OF THE CONSTANTS a AND b FOR THE BINDING OF ALKALI BY DURUMIN

N $\times 10^{-3}$	log N	n $\times 10^{-3}$	log n	x^2	xy
5	0.6990	2	0.3010	0.4886	0.2119
10	1.0000	3	0.4771	1.0000	0.4771
20	1.3010	4	0.6021	1.6926	0.7833
30	1.4771	5	0.6990	2.1818	1.0325
40	1.6021	8	0.9031	2.5667	1.4468
50	1.6990	10	1.0000	2.8866	1.6990
60	1.7782	15	1.1761	3.1620	2.0193
80	1.9031	21	1.3222	3.6218	2.5163
100	2.0000	27	1.4314	4.0000	2.8628
120	2.0792	34	1.5315	4.3231	3.1843
140	2.1461	33	1.5185	4.6057	3.2588
160	2.2041	34	1.5315	4.8580	3.3756
200	2.3010	54	1.7324	5.2946	3.9862
250	2.3979	79	1.8976	5.7499	4.5502
300	2.4771	83	1.9191	6.1360	4.7538
350	2.5441	89	1.9494	6.4724	4.9595
400	2.6021	97	1.9868	6.7709	5.1698
450	2.6532	126	2.1004	7.0394	5.5728
500	2.6990	129	2.1106	7.2846	5.6965
	37.5633		26.1898	80.1350	57.6287

$$a = \frac{37.5633 \times 57.6287 - 80.1350 \times 26.1898}{(37.5633)^2 - 19 \times 80.1350}$$

$$= \frac{2164.7247 - 2098.7196}{1411.0015 - 1522.5650}$$

$$= -0.5901$$

$$b = \frac{37.5633 \times 26.1898 - 19 \times 57.6287}{(37.5633)^2 - 19 \times 80.1350}$$

$$= \frac{983.7753 - 1094.9456}{1411.0015 - 1522.5650}$$

$$= 0.9957$$

sive increase in the amount of acid or alkali bound by the protein as the original acid or alkali concentration was increased. At the higher concentrations the ratio between the amount of acid or alkali added and the amount bound by the protein decreased.

It has been shown by Isaquiure (1923) that for gelatin and hydrochloric acid, the logarithms of the acid bound plotted against the

TABLE C

CONSTANTS FOR THE LOGARITHMIC CURVE, $y = a + bx$, FOR ACID AND ALKALI BINDING OF VARIOUS PROTEINS WHERE y = LOG OF ACID OR ALKALI BOUND AND x = LOG OF ACID OF ALKALI ADDED.

Proteins	Sodium hydroxide		Hydrochloric acid		Sulfuric acid		Phosphoric acid	
	a	b	a	b	a	b	a	b
Gliadin	−0.4111	0.9563	−0.2007	0.8118				
Speltin	−0.5630	0.9831	−0.3053	0.8297				
Durumin	−0.5901	0.9957	−0.1372	0.7786	−0.1253	0.7854	−0.2571	0.8917
Dicoccumin	−0.5754	1.0000	−0.2886	0.8476				
Monococcumin	−0.5611	0.9833	−0.1766	0.8072				
Secalin	−0.3857	0.9333	−0.1263	0.8466				
Sativin	−0.2361	0.8752	−0.5094	0.9439				
Hordein	−0.4403	0.9914	−0.3122	0.8858				
Zein	−0.2570	0.8540	−0.3136	0.9291				
Teozein	−0.2585	0.8666	−0.3229	0.8971				
Kafirin	−0.3723	0.9642	−0.4989	0.9152				
Sorghumin	−0.1340	0.8864	−0.5643	0.9158				
Casein	+0.0738	0.7549	+0.0489	0.6676	−0.0378	0.6851	−0.0889	0.8549
Fibrin	+0.0192	0.7934	+0.1644	0.7166				

logarithms of the original acid concentration form a straight line. The logarithms of the amount of acid bound by the various proteins was accordingly plotted against the logarithms of the original acid concentration and straight lines were obtained. These lines were so nearly identical that it was impossible to plot the curves for the various proteins for to do so would necessitate a special graph for each protein.

The formula for a straight line plotted from logarithms is:

$$\log y = a + b\ (\log x).$$

Where a and b are constants, a determines the position of the line on the axis of abscissa at zero concentration of acid or alkali and b is the tangent of the angle which the line makes with the horizontal, in other words b is directly related to the slope of the line. In order to give values from which the lines could be reproduced, the constants a and b of the formula for a straight line, where $y = \log$ of acid or alkali bound, and $x = \log$ of the original concentration of acid or alkali, were calculated by the method of least squares as given by Mellor (1916) where:

$$a = \frac{\Sigma(x) \cdot \Sigma(xy) - \Sigma(x^2) \cdot \Sigma(y)}{[\Sigma(x)]^2 - n\,\Sigma(x^2)}$$

$$b = \frac{\Sigma(x) \cdot \Sigma(y) - n\,\Sigma(xy)}{[\Sigma(x)]^2 - n\,\Sigma(x^2)}$$

An example of the calculation for the binding of sodium hydroxide by durumin, showing the detailed procedure, is given in Table XCIX.

An examination of these constants as given in Table C, shows close relationship between the proteins of the wheat series and between the proteins of the maize series although there is a marked difference between the two groups. One important fact brought out when these values are substituted in the formula is that regardless of the values of a and b, the values for y are almost identical at the higher values of x.

Fig. 6 for hydrochloric acid and Fig. 7 for sodium hydroxide show these curves reproduced from the constants of casein and durumin.

If the values for the acid and alkali bound are plotted according to the method used by Loeb and coworkers, the final pH is the abscissa and the equivalents of acid or alkali bound, the ordinates. This type of curve obtained for hydrochloric acid is shown in Fig. 8. The graph shows that the amount of acid bound does not become constant as the hydrogen ion concentration increases but continues to increase. At first sight it appears that the amount of acid bound in proportion to the final hydrogen ion concentration is increasing very rapidly but in reality it is decreasing. This type of curve is the result of plotting logarithms against natural values. Figs. 4 and 8 are drawn from the

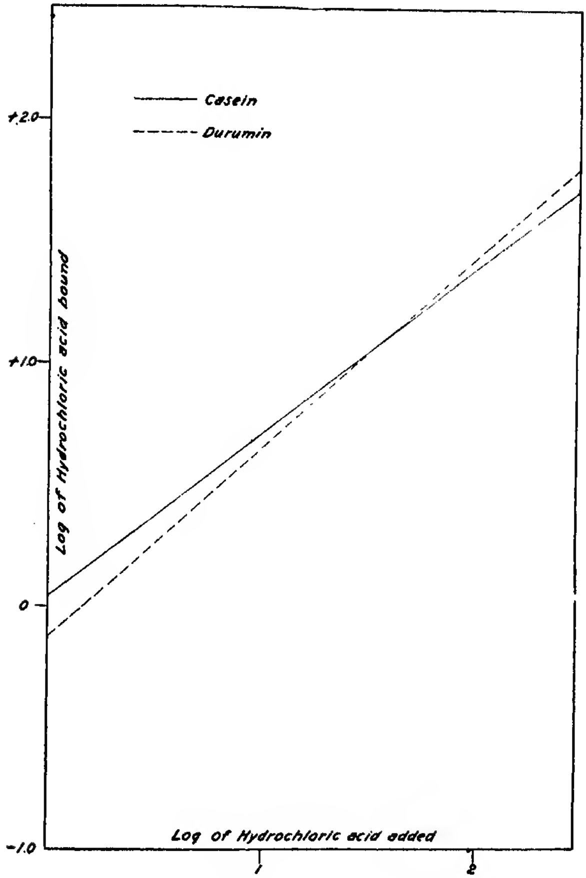


FIG. 6.—The relation of the logarithms of the "acid added" to "acid bound" by durumin and casein. (Note that the data are for hydrogen ion concentrations in excess of the equivalent of pH 2.5.)

same data except that the abscissa in Fig. 4 is the original normality of the acid while in Fig. 8 it is the final pH. If, in the latter case, the original pH had been used, the same type of curve as in Fig. 8 would have resulted.

When the logarithms of the equivalents of acid or alkali bound by the protein are plotted against the final pH a straight line is also

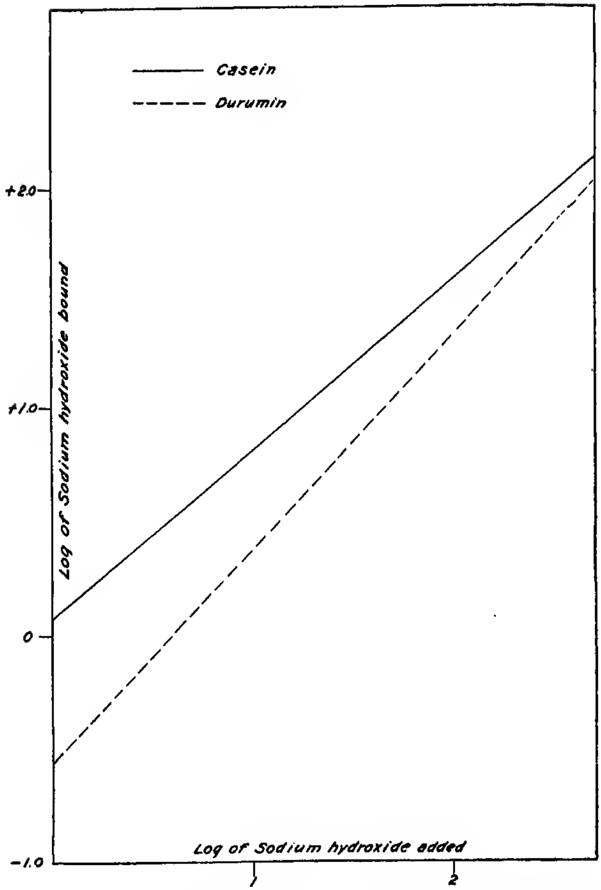


FIG. 7.—The relation of the logarithms of the "alkali added" to "alkali bound" by durumin and casein. (Note that the data are for hydroxyl ion concentrations in excess of the equivalent of pH 10.5.)

formed. The constants *a* and *b* were calculated by the method of least squares, where x = the final pH and y = the log of the equivalents of acid or alkali bound by the protein. These values are given in Table CI. Almost the same values were obtained for *b* as in Table C, but the values for *a* are different inasmuch as different values were used for *x*.

TABLE CI

CONSTANTS FOR THE LOGARITHMIC CURVE, $y = a + bx$, FOR THE ACID AND ALKALI BINDING OF THE VARIOUS PROTEINS WHERE y = THE LOG OF THE ACID OR ALKALI BOUND AND x = THE EQUILIBRIUM pH.

	Hydrochloric acid		Sodium hydroxide	
	a	b	a	b
Gliadin	2.2612	—0.7156	—10.3681	0.9302
Speltin	2.2890	—0.8009	—11.9498	1.0545
Durumin	2.2354	—0.6929	—11.7861	1.0400
Dicoccumin	2.3422	—0.8039	—10.8334	0.9683
Monococcumin	2.2558	—0.6921	—11.1013	0.9858
Secalin	2.4466	—0.7502	—9.2751	0.8513
Sativin	2.5024	—0.9660	—9.4752	0.8666
Hordein	2.3964	—0.8003	—10.5823	0.9629
Zein	2.6894	—0.9369	—8.9214	0.8252
Teozein	2.3782	—0.7809	—8.2096	0.8209
Kafirin	2.4307	—0.9613	—11.1521	1.0071
Sorghumin	2.3420	—0.9537	—10.9363	1.0000
Casein	1.9805	—0.5062	—5.7020	0.5819
Fibrin	2.2438	—0.5209	—3.7158	0.4399

Comparison of Hydrochloric Acid, Sulfuric Acid and Phosphoric Acid Binding. The amounts of hydrochloric and sulfuric acid bound by durumin and casein when compared on the original normality basis are almost identical and but slightly lower than for phosphoric acid (molar). Approximately the same amount of each acid was bound by both proteins. Fig. 4 shows the curves obtained for these values. The points do not fall on a smooth line but if all the values were corrected for the probable experimental error, a smooth curve with all of the points falling on it would be obtained.

The equivalents of acid bound by the proteins was also plotted against the final pH as shown in Fig. 8. Here a difference between the three acids is brought out. The proteins bind less hydrochloric acid at a definite pH than sulfuric acid and less sulfuric acid than phosphoric acid.

According to Loeb and Hitchcock, the same amount of hydrochloric, sulfuric and phosphoric acid are bound at the same pH by a protein. The results given above do not agree with this view. In the case of Loeb's results for the hydrochloric, sulfuric and phosphoric acid binding by albumin he found that the same quantity of each acid (considering phosphoric acid as monovalent) was required to bring equal amounts of the protein to the same pH. Concerning this he states (Loeb, 1922, Fig. 4, page 44), "Beginning with the lowest curve, we notice that the curve is the same for 0.1 N HCl and 0.1 N H_2SO_4, since both are strong acids; or, in other words, H_2SO_4 combines in equivalent proportions with egg albumin. The curve for H_3PO_4 is the highest curve (where cubic centimeters of 0.1 N acid in 100 cc. of a

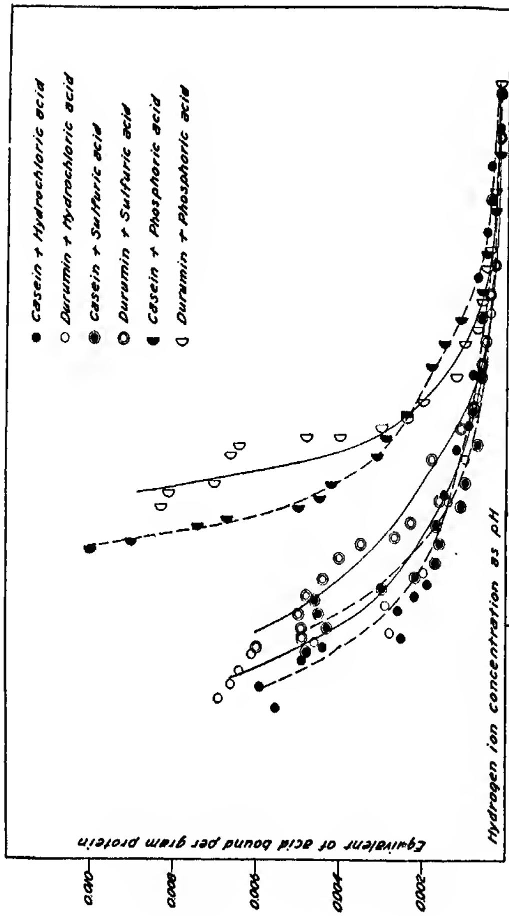


FIG. 8.—The relation of the equivalents of acid bound to the equilibrium pH.

one per cent solution of isoelectric albumin is plotted as ordinates and the equilibrium pH is the abscissa) and if we compare the values for H_3PO_4 with those for HCl (or H_2SO_4) we notice that for each pH, the ordinate for H_3PO_4 is nearly three times as high as that for HCl as the accuracy of our experiment permits. This means that H_3PO_4 combines with albumin (inside the range of pH of our experiment) in molecular proportions and that the anion of albumin phosphate is the monovalent anion H_2PO_4."

Loeb found that the same relationship holds when the amount of acid bound by the protein is calculated by the difference in the number of cubic centimeters of acid required to bring (1) the same volume of water alone, and (2) the same volume of protein solution, to the same pH. In a discussion of these results he states, "Figure 6 contains the curves whose ordinates give the amount of cc. of 0.1 N HCl, H_2SO_4, $H_2C_2O_4$, and H_3PO_4 in combination with one gram of originally isoelectric egg albumin at different pH. It appears again that the curves for HCl and H_2SO_4 practically coincide as the purely chemical theory demands, that the oxalic acid curve is higher, and that the H_3PO_4 curve is still higher. What is of greater importance is that for the same pH the ordinates of the H_3PO_4 curve are always approximately three times as high as the ordinates of the curves for HCl and H_2SO_4." Here again, if a molar solution of phosphoric acid is considered as an equivalent solution, Loeb argues that one gram of protein binds equivalent amounts of hydrochloric, sulfuric and phosphoric acid at the same pH.

The data presented by Loeb indicate that the same quantity of 0.1 normal hydrochloric and sulfuric acid and molar phosphoric acid give the same equilibrium pH when equal amounts of protein is added to each acid and that when equilibrium is reached the protein has bound equal amounts of each acid. The only conditions under which this could be possible are that equivalent solutions of the three acids give the same hydrogen ion concentration or that the residual or free acid does not ionize the same in the protein solution as in pure water. The latter condition is hardly probable and further, the assumption on which his calculations are made is that the same concentration of acid in water and in protein solution gives the same hydrogen ion concentration as measured potentiometrically. The former condition does not hold, for, the degree of ionization, given in the preceding section, of phosphoric acid as well as the values given by Hitchcock (1922) is only about 25 per cent that of equivalent concentrations of hydrochloric acid. To demonstrate this the values given by Loeb (1922) for the binding of acids by gelatin serve as a specific example. He found that 20 cc. of 0.1 normal phosphoric acid were required to bring one gram of gelatin in 100 cc. solution to pH 3.2 at which pH 18 cc. of 0.1 normal phosphoric acid were bound by the gelatin. This leaves only 2 cc.

of 0.1 normal phosphoric acid in 100 cc. or a 0.002 normal solution. If the phosphoric acid is considered monovalent the solution is only 0.0007 normal. In the case of hydrochloric acid 7.5 cc. of 0.1 normal acid were required to bring 100 cc. of 1 per cent gelatin solution to pH 3.2 and at this hydrogen ion concentration, the gelatin bound 6.5 cc. of normal acid, this leaves an equilibrium concentration of 0.001 normal hydrochloric acid. If these figures are correct, phosphoric acid would have to be considered a stronger acid than hydrochloric acid. Experimental evidence shows the "ionization" of hydrochloric acid to be from 4 to 5 times greater than phosphoric acid at the same (molar) concentration.

Further evidence that Loeb's assumptions concerning the ratios of different acids bound by proteins are not correct is furnished by Hitchcock (1922). He showed that when the amount of phosphoric acid bound by edestin is calculated by the same method employed by Loeb, there is about 5 times as much 0.1 normal phosphoric as hydrochloric acid bound. He was able to get equal amounts of hydrochloric and phosphoric (molar solution) acid bound by edestin when the amount of phosphoric acid was calculated from the ionization constant and the amount of hydrochloric acid calculated in the usual manner from potentiometric measurements.

Loeb's experiments which show that equal amounts of hydrochloric and phosphoric acid bring a protein solution to the same pH and that a protein binds equal amounts of the two acids at the same pH serve as his principal evidence on which he "proved" that acids and alkali combine with proteins in a stoichiometric relationship. Experiments reported in this paper as well as by Hitchcock support the view that equal amounts of a protein do not bind equal amounts of hydrochloric and phosphoric acid at the same pH. The data presented in the previous section of this paper show that approximately the same amount of normal hydrochloric and sulfuric acids and only slightly more (molar) phosphoric acid are bound by equal amounts of protein when the comparisons are made on the basis of the original acid concentration. *When the comparisons are made on the basis of the final hydrogen ion concentration expressed in pH, approximately the same amount of sulfuric and hydrochloric acid but considerably more phosphoric acid is bound.* This is in agreement with the results reported by Pauli and Hirschfeld (1914) for the binding of acetic acid by proteins.

The constants *a* and *b* were also calculated using the logarithms of acid bound as y and the log of the original concentration of acid as x. The results are given in Table C. The values for the logarithms of the equivalents of acid bound as y and the original pH as x are given in Table CII, and for the logarithms of the equivalents of acid bound as y and the final pH as x are given in Table CIII. A corresponding although greater increase in the values of *b* is noted. This is not as

TABLE CII

CONSTANTS FOR THE LOGARITHMIC CURVE, $y = a + bx$, FOR ACID BINDING OF VARIOUS PROTEINS WHERE y = LOG OF ACID BOUND AND x = pH OF THE ORIGINAL ACID CONCENTRATION

	Hydrochloric acid		Sulfuric acid		Phosphoric acid	
	a	h	a	h	a	h
Durumin	2.2678	—0.7685	2.5907	—0.9209	4.0147	—1.4173
Casein	2.1219	—0.7039	2.2891	—0.7832	3.5320	—1.2786

TABLE CIII

CONSTANTS FOR THE LOGARITHMIC CURVE, $y = a + bx$, FOR ACID BINDING OF VARIOUS PROTEINS WHERE y = LOG OF ACID BOUND AND x = pH OF THE FINAL ACID CONCENTRATION

	Hydrochloric acid		Sulfuric acid		Phosphoric acid	
	a	b	a	h	a	h
Durumin	2.2354	—0.6929	2.4154	—0.7741	3.4886	—1.0642
Casein	1.9805	—0.5062	2.1002	—0.5843	3.0459	—0.9835

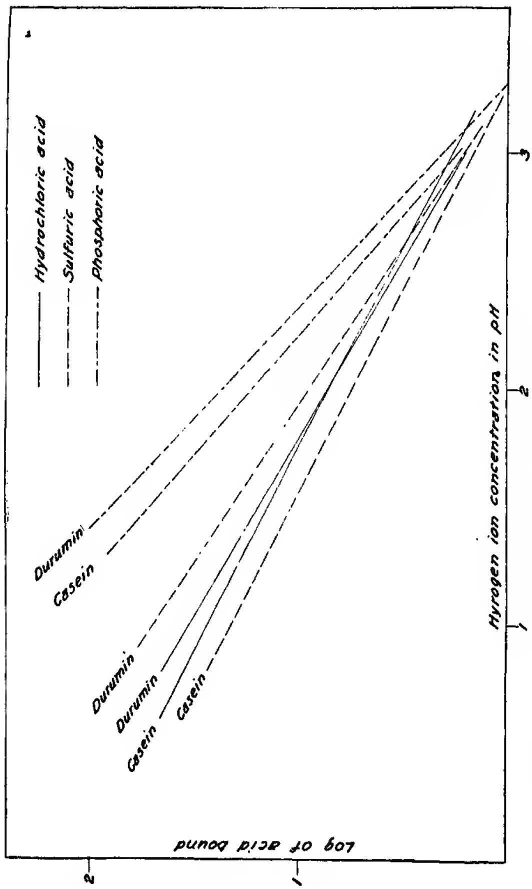


FIG. 9.—The data shown in Fig. 8 plotted as logarithms of equivalents of acid bound against the equilibrium pH.

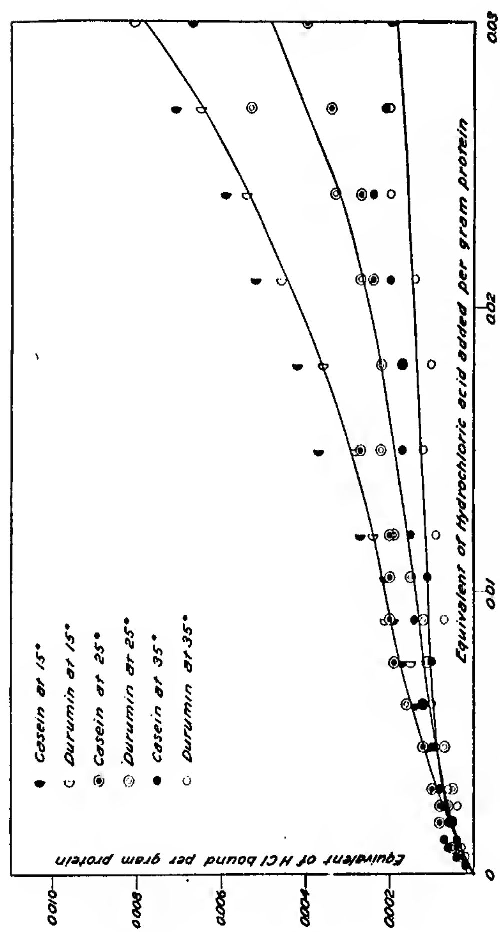


FIG. 10.—Temperature curves of the relation of hydrochloric acid added to hydrochloric acid bound by durumin and casein. Only the lines for durumin are drawn.

significant as might first appear as the values for *a* are also increasing so that the lines approach each other. The values for the logarithms of acid bound as y and the final pH as x, Table CIII, are similar to those in Table CI. The curves plotted from the constants *a* and *b*, Table CIII, are shown in Fig. 9.

The Temperature Coefficients of Acid and Alkali Binding. The results obtained by the potentiometric titration of proteins at 15°, 25°, and 35° C. show that temperature has a decided effect on the amount of acid or alkali bound by a protein especially at the higher concentrations of acid or alkali.

TABLE CIV

CONSTANTS FOR THE LOGARITHMIC CURVE, $y = a + bx$, FOR THE ACID OR ALKALI BINDING OF VARIOUS PROTEINS, WHERE y = THE LOG OF EQUIVALENTS OF ACID OR ALKALI BOUND AND x = THE LOG OF THE ORIGINAL CONCENTRATION

	Hydrochloric acid		Sodium hydroxide	
	a	b	a	b
Durumin 15°	−0.5628	0.9627	−0.7012	1.0621
Durumin 25°	−0.1733	0.6981	−0.4680	0.8755
Durumin 35°	−0.0348	0.5414	−0.3860	0.7372
Teozein 15°	−0.5258	0.9586	−0.5540	1.0317
Teozein 25°	−0.5493	0.8644	−0.2366	0.8752
Teozein 35°	−0.5053	0.8013	−0.1651	0.6164
Casein 15°	−0.2421	0.7468	−0.0918	0.8263
Casein 25°	+0.1128	0.5845	+0.2363	0.6427
Casein 35°	+0.2044	0.4658	+0.4630	0.4467
Fibrin 15°	+0.0147	0.6659	+0.0605	0.7770
Fibrin 25°	+0.3102	0.4856	+0.0782	0.7354
Fibrin 35°	+0.4115	0.4498	+0.2613	0.5239

Figs. 10 and 11, where the equivalents of acid or alkali bound are plotted as ordinates and the original concentration as abscissa show that the ratio of the amount of acid or alkali bound is inversely proportional to the difference in temperature. This is in approximately the ratio of 1:2:3 where 1 is 35°. This is not the rate that would be expected if the binding is a chemical process but is that which would be expected if the binding is an adsorption phenomenon or obeys the purely physical adsorption law.

The amount of acid bound at 35° by the proteins used is in the range found by Hitchcock (1923) for gelatin and hydrochloric acid for similar concentrations of acid. *In any attempt to correlate the "maximum" amount of acid cr alkali bound by a protein with the chemical composition of the protein both the final acid concentration and the temperature must be taken into account.*

When the constants for *a* and *b* were calculated using the logarithms of the acid or alkali bound as y and the logarithms of the original acid

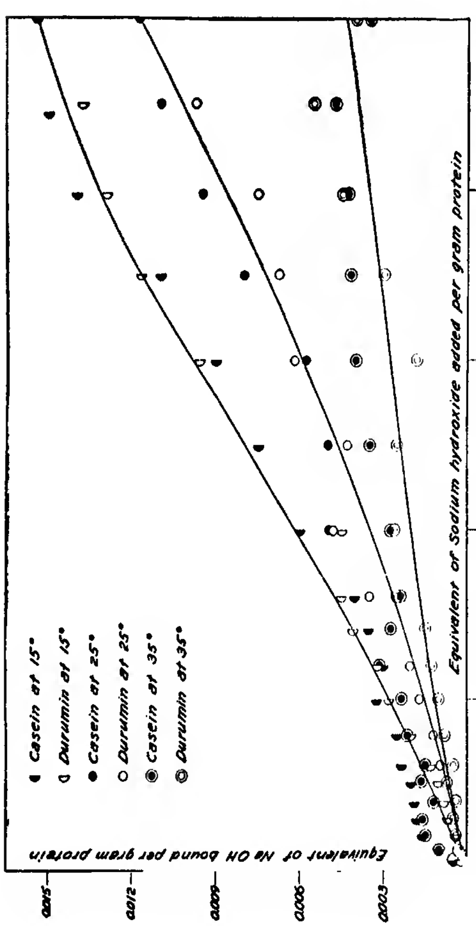


FIG. 11.—Temperature curves of the relation of sodium hydroxide added to sodium hydroxide bound by durumin and casein. Only the lines for durumin are drawn.

or alkali concentration (Table CIV) or the equilibrium pH (Table CV) as x, the values for *b* change inversely as the temperature. The values for *a* show a similar change.

TABLE CV

CONSTANTS FOR THE LOGARITHMIC CURVE, $y = a + bx$, FOR THE ACID OR ALKALI BINDING OF VARIOUS PROTEINS, WHERE y = THE LOG OF EQUIVALENTS OF ACID OR ALKALI BOUND AND x = THE pH OF THE EQUILIBRIUM SOLUTION

	Hydrochloric acid		Sodium hydroxide	
	a	b	a	b
Durumin 15°.........	2.3232	—0.8268	—13.2329	1.1586
Durumin 25°.........	1.8547	—0.5837	—9.6837	0.8668
Durumin 35°.........	1.4743	—0.4352	—8.0818	0.7190
Teozein 15°.........	2.6729	—1.0031	—12.2190	1.0886
Teozein 25°.........	2.1227	—0.8721	—8.0700	0.7594
Teozein 35°.........	1.9212	—0.7830	—7.1225	0.6589
Casein 15°.........	2.1228	—0.6041	—6.3836	0.6349
Casein 25°.........	1.7961	—0.4601	—4.5818	0.4852
Casein 35°.........	1.4954	—0.3373	—3.8993	0.4153
Fibrin 15°.........	2.0842	—0.4891	—4.8892	0.5207
Fibrin 25°.........	1.6594	—0.3316	—4.3239	0.4670
Fibrin 35°.........	1.7613	—0.3659	—3.6002	0.3887

When the lines are plotted from the constants given in Table CIV, as shown in Figs. 12 and 13 for acid and Figs. 14 and 15 for alkali, it is noted that the three lines representing the values for 15°, 25°, and 35°, for a particular protein cross at approximately the same point. This holds true for both hydrochloric acid and sodium hydroxide and for all four proteins used in this experiment. For hydrochloric acid these points represent a final pH of about 2.5 and for sodium hydroxide a final pH of about 10.5.

The data show that when the hydrogen ion concentration is greater than about pH 2.5 or the hydroxyl ion concentration is more than about pH 10.5 there is a marked negative temperature coefficient, *i.e.*, there is less acid bound at the higher temperatures. The values obtained from extrapolating the lines below the point where they cross cannot be used. We wish again to call attention to the fact that the constants *a* and *b* were calculated from the data where the equilibrium hydrogen ion concentration was *greater than pH 2.5* and the equilibrium hydroxyl ion concentration was *greater than pH 10.5*. A discussion of the portion of the curves lying between pH 2.5 and pH 10.5 will be taken up later.

These points (pH 2.5 and 10.5) appear to be the hydrogen and hydroxyl ion concentrations at which some distinct change in the character of the acid or alkali binding reaction takes place. Proteins show maximum swelling, maximum viscosity and maximum surface tension

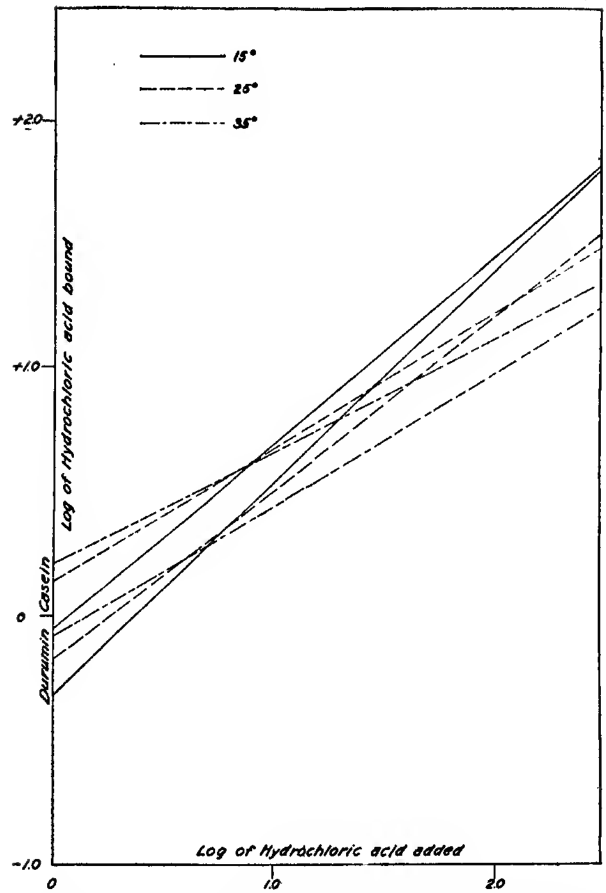


FIG. 12.—The relation of the logarithms of the acid added to the acid bound by durumin and casein at 15°, 25°, and 35° C. (Note that the data are for equilibrium hydrogen ion concentrations in excess of the equivalent of pH 2.5.) The portion of the lines to the left of the point of intersection is obtained by extrapolation and does not hold experimentally.

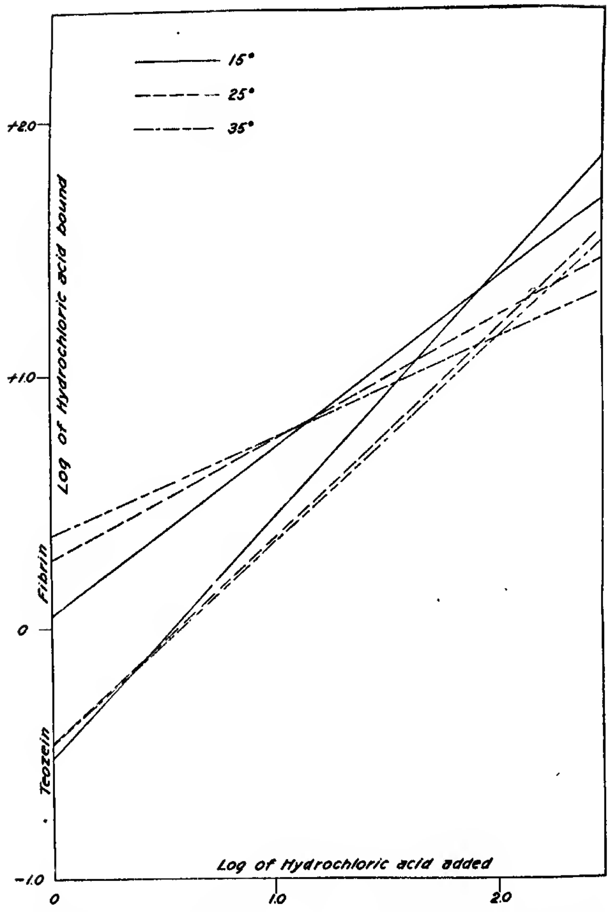


FIG. 13.—The relation of the logarithms of the acid added to the acid bound by teozein and fibrin at 15°, 25°, and 35° C. (Note that the data are for equilibrium hydrogen ion concentrations in excess of the equivalents of pH 2.5.) The portion of the lines to the left of the point of intersection is obtained by extrapolation and does not hold experimentally.

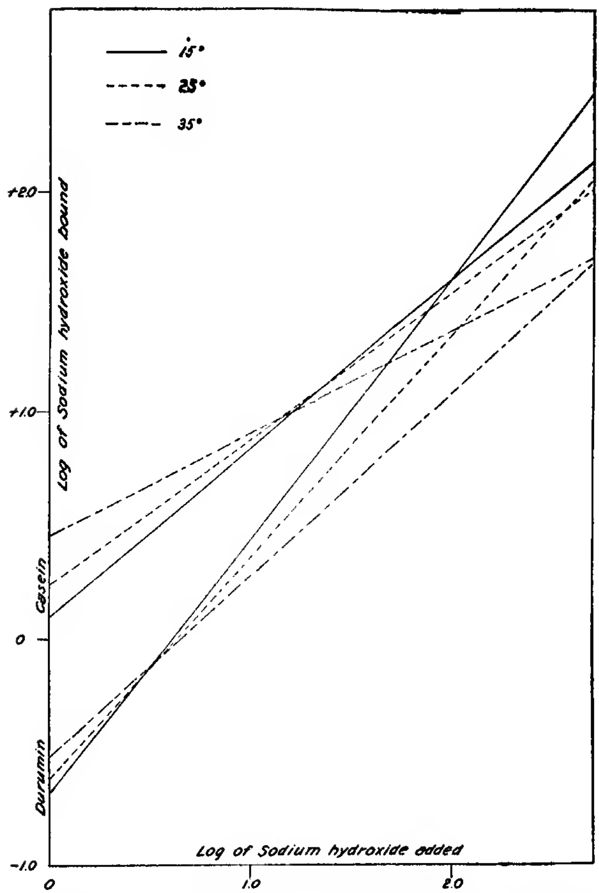


FIG. 14.—The relation of the logarithms of the alkali added to the alkali bound by durumin and casein at 15°, 25°, and 35° C. (Note that the data are for equilibrium hydroxyl ion concentrations in excess of the equivalent of pH 10.5.) The portion of the lines to the left of the point of intersection is obtained by extrapolation and does not hold experimentally.

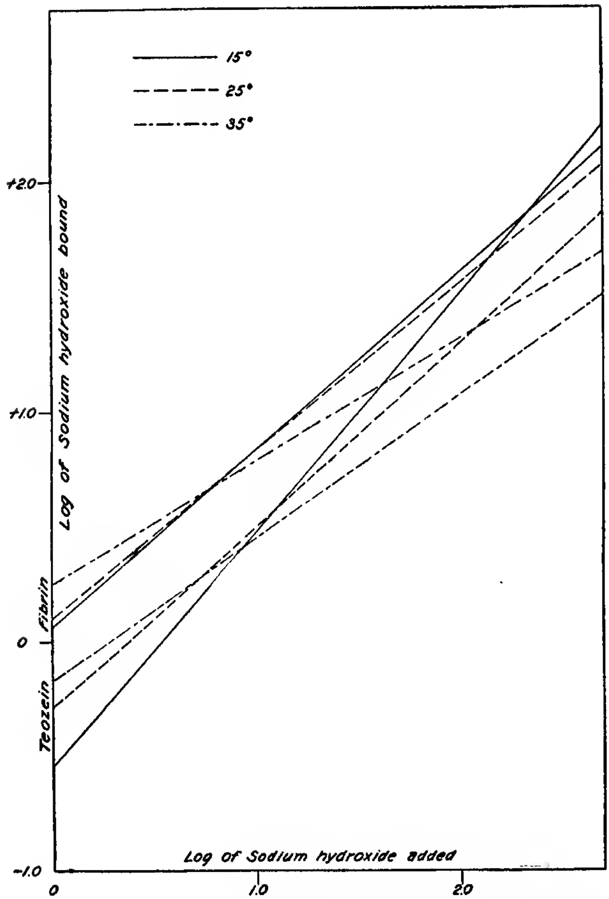


FIG. 15.—The relation of the logarithms of the alkali added to the alkali bound by teozein and fibrin at 15°, 25°, and 35° C. (Note that the data are for equilibrium hydroxyl ion concentrations in excess of the equivalents of pH 10.5.) The portion of the lines to the left of the point of intersection is obtained by extrapolation and does not hold experimentally.

at these hydrogen ion concentrations when acid or alkali is added to isoelectric protein. Pauli postulates that this (pH 2.5) is the point where complete ionization of the protein-acid salt takes place.

The Nature of Acid and Alkali Binding Between pH 2.5 and pH 10.5. When the final hydrogen ion concentration was not more than about pH 2 or the hydroxyl ion concentration more than about pH 11, the amount of acid or alkali bound by proteins is the same for 22° and 35°, in other words, a marked temperature coefficient is no longer apparent.

If the buffer curves, Fig. 16, are plotted with the cubic centimeters of acid or alkali added as ordinates and the final pH as abscissa, a significant difference is observed between the curves for durumin and casein. On the acid side the difference is not as great as on the alkaline side. The first 0.1 cc. of normal sodium hydroxide causes only a slight change in the pH of the casein solution but the change is about 3 on the pH scale in the durumin solution.

There is a break in the curves at about pH 2.5 on the acid side and about pH 10.5 on the alkaline side. If the difference in the number of cubic centimeters of sodium hydroxide or hydrochloric acid required to bring casein and durumin to these hydroxyl or hydrogen ion concentrations were subtracted from the total added to the casein solution at the higher concentrations of acid or alkali, the lines for the two proteins on the acid side of pH 2.5 and on the alkaline side of pH 10.5 would superimpose. This substantiates the evidence produced in Figs 12-15 inclusive that at about pH 2.5 and pH 10.5 there is a change in the type of combination between the protein and acid or alkali. Above this hydrogen or hydroxyl ion concentration there is probahly a physical combination or at least the combination follows the adsorption laws. Between these values there is probably a true chemical combination.

Curves, Figs. 17 and 18, similar to those in Figs. 4 and 5, were obtained when the amount of acid or alkali bound was plotted as ordinates and pH as abscissa. The difference between the various proteins is not as marked in the case of acid as in the case of alkali. These curves likewise show a break at ahout pH 2.5 and pH 10.5 (Figs. 12-15). The first addition of sodium hydroxide, as in the buffer curve, brings about very little change in the pH of the casein and fibrin solutions but causes a definite change in the pH of the teozein and durumin solutions.

In Fig. 17, the behavior of durumin towards acid is rather peculiar. First there is a rapid increase in the amount of acid bound and then almost no increase until the hydrogen ion concentration reaches pH 2.5 when more acid is bound. The binding of alkali, by the various proteins, Fig. 18, likewise shows peculiarities. The durumin and teozein solutions reach a hydroxyl ion concentration of pH 10.5 at

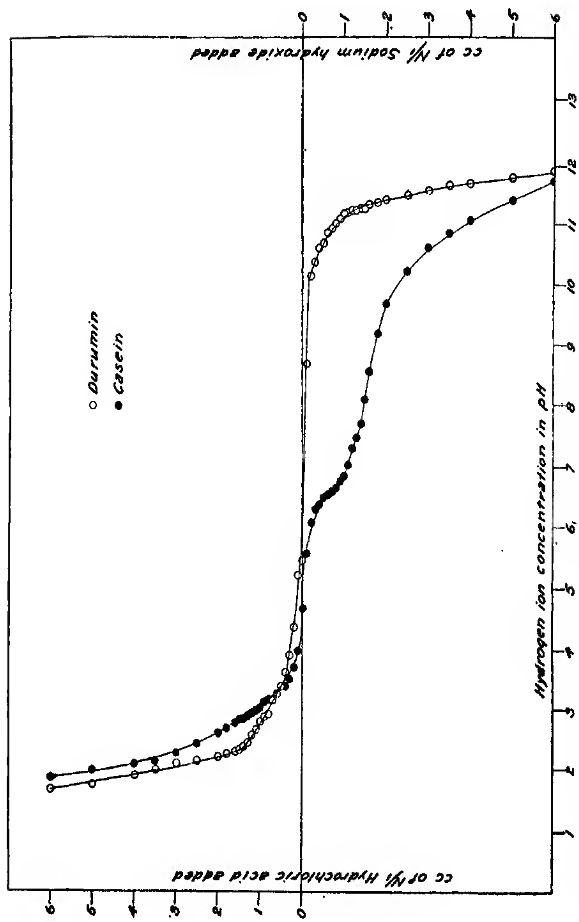


FIG. 16.—Buffer curves of durumin and casein in various concentrations of hydrochloric acid and sodium hydroxide.

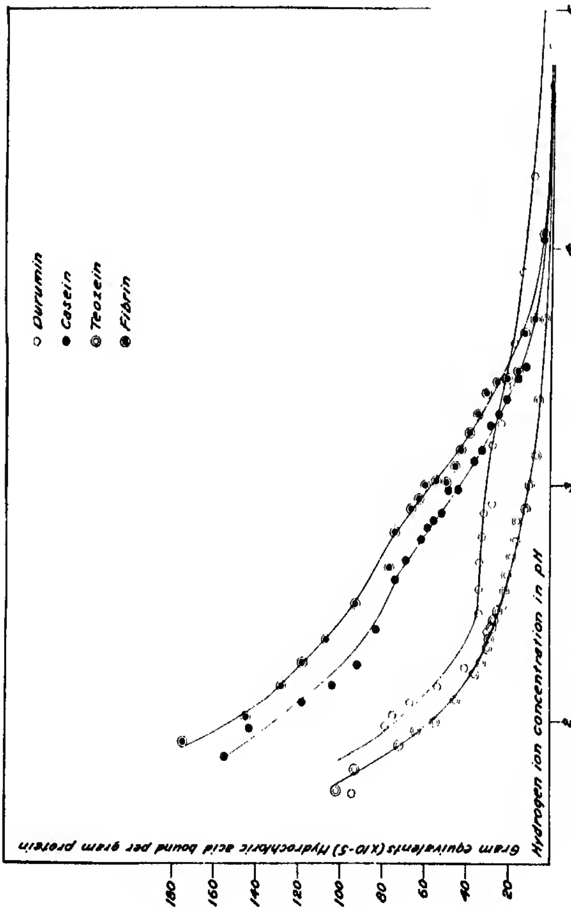


FIG. 17.—The relation of the equivalents of acid bound to the equilibrium pH.

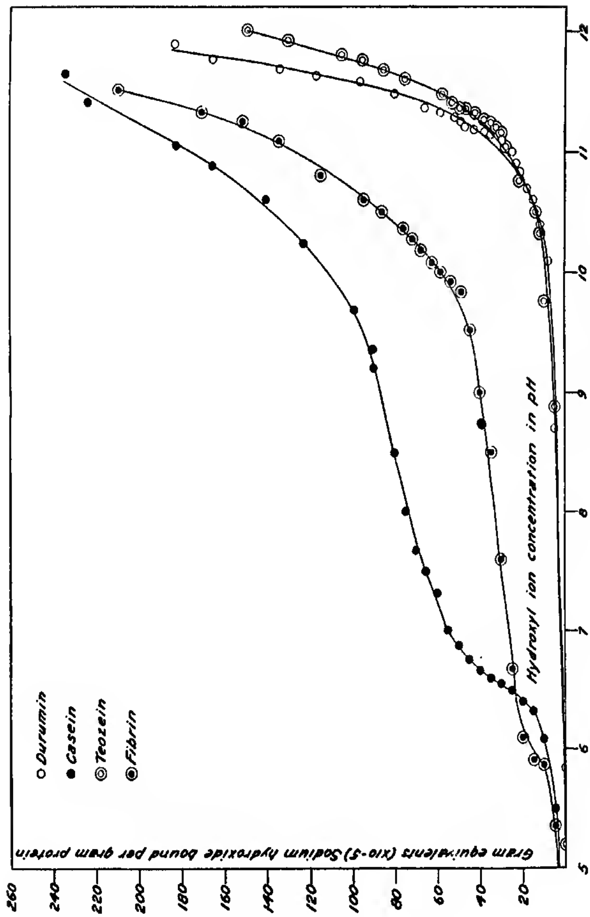


FIG. 18.—The relation of the equivalents of alkali bound to the equilibrium pH.

extremely low concentrations of added alkali while above this hydroxyl ion concentration both proteins bind additional sodium hydroxide. Fibrin binds considerable alkali at the lower hydroxyl ion concentrations but the curve soon flattens and not much more is bound until pH 10.5 is reached beyond which point the amount bound increases rapidly. The casein curve is distinctly different from the other three. The amount of sodium hydroxide bound gradually increases until about pH 7 and then the curve flattens and not much more alkali is bound until the hydroxyl ion concentration reaches about pH 10.5. This peculiar behavior must be due to some acid group in casein which is apparently absent in the prolamines although somewhat in evidence in fibrin.

These data indicate that there must be two types of combination between proteins and acid or alkali. One type, a chemical type of combination, takes place below a hydrogen ion concentration of cH *0.315 x* 10^{-2} *(pH 2.5) and below a hydroxyl ion cancentration of abaut* $cOH = 3.32 \times 10^{-4}$ *(pH 10.5). The other type, an adsorption type of combination, takes place above a hydrogen ion concentration of about pH 2.5 and above a hydroxyl ian concentration af about pH 10.5.*

Evidence of the second type of binding is furnished by:

1. At the higher concentrations of acid and alkali, all of the proteins used in this work *regardless of their chemical composition,* bind at equal protein concentrations approximately the same amount of acid or of alkali.
2. The negative temperature coefficient of the acid or alkali binding at the higher concentrations also furnishes evidence of an adsorption phenomenon.
3. The logarithms of the amount of acid or alkali bound plotted against the logarithms of the original acid or alkali concentration or against the final pH, form a straight line.
4. There is more alkali bound when the original concentration is 0.5000 normal than can be accounted for by chemical combination assuming that there is an available carboxyl group for each nitrogen atom, an assumption far in excess of possibility.
5. When the hydrogen ion concentration is greater than about pH 2.5 there is no increase in the ionization of the protein chloride. (*Cf.* Pauli, and Hitchcock.)

Evidence of a chemical type of combination between a hydrogen ion concentration of pH 2.5 and 10.5 is presented by:

1. The logarithms of the amount of acid or alkali bound plotted against the original concentrations do not form a straight line.
2. The buffer curves do not form a smooth, regular line.
3. The amount of acid or alkali bound at any hydrogen ion

concentration between pH 2.5 and 10.5, depends on the chemical composition of the protein. This is not true where the pH is less than 2.5 or greater than 10.5.

4. When the hydrogen ion concentration is below about pH 2.5, the protein chloride is highly ionized. (*Cf.* Pauli, and Hitchcock.)

Protein Groups Responsible for Chemical Binding of Acids and Alkalies. The active protein groups combining with acid have been considered by Greenberg and Schmidt (1924) and by Hitchcock (1923) to be the free amino group of lysine and the amino group of the guanidine nucleus of arginine which does not react with nitrous acid. For alkalies, Greenberg and Schmidt consider that the binding is at the free carboxyl groups of glutamic and aspartic acids, β-hydroxy-glutamic acid and the hydroxyl group of tyrosine.

Due to the insufficient data on the content of these amino acids in proteins, it is difficult to correlate between their free acid groups and the amount of alkali bound at a certain hydroxyl ion concentration. In Table CVI, an attempt was made to collect the available data on some of the proteins to ascertain any relationship which might exist between the alkali binding and the chemical constitution. *There is no correlation between the quantity of alkali bound at a definite hydroxyl ion concentration and the content of the divalent amino acids in the proteins as given in the literature.* Casein is the only protein that shows a close relationship between the calculated amount of sodium hydroxide which should be bound at pH 10.5 and the amount actually bound. Fibrin binds much more alkali than it should from the data on the amino acid content, while *zein and hordein bind much less than the calculated amount.* Greenberg and Schmidt (1924) report that gliadin binds 30×10^{-5} equivalents of alkali at pH 11 and that the calculated value is 34×10^{-5} equivalents. If, however, they had used the value 1.24 per cent for tyrosine (Abderhalden and Samuely, 1905) instead of 0.2 per cent (Osborne and Guest, 1911a) the calculated value would have been 43×10^{-5}. This increases their error between the observed and calculated values from 13.3 per cent to 43.3 per cent. Until more reliable analyses for these amino acids in proteins are available or until *exact* measurements of free acidic groups are carried out it will be impossible to draw any exact conclusions as to the relationship between the composition of the protein and the amount of alkali bound at a certain hydroxyl ion concentration.

In the case of acid binding, the groups which are supposed to bind acid can be readily determined. It is assumed that the seat of the chemical binding of acid is at the free amino groups of the native protein and at the amino group of the guanidine nucleus of arginine which does not react with nitrous acid. Both of these groups can be

TABLE CVI

THE BASE-COMBINING POWER OF SOME OF THE PROTEINS

	Glutamic acid	Aspartic acid	β-hydroxy-glutamic acid	Tyrosine	Ammonia nitrogen	Total acidic groups	Free acidic groups [12]	Gm. equiv. NaOH bound per gm. protein at pH 10.5 [3]
						$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$
Molecular weight	147	133	163	181	14			
Gliadin,						349	41	20
Amino acid content, per cent.......	43.7 [1]	1.24 [2]	2.4 [11]	5.1 [10]	4.31 [1]			
Amino acid content gm. equi. $\times 10^{-5}$	297	9	15	28	308			
Secalin,						238	—25	25
Amino acid content, per cent.......	33.8 [4]	0.25 [4]		1.19 [4]	3.68 [3]			
Amino acid content gm. equi. $\times 10^{-5}$	230	2		6	263			
Hordein,						314	61	25
Amino acid content, per cent.......	41.3 [5]	1.32 [5]		4.00 [5]	3.54 [5]			
Amino acid content gm. equi. $\times 10^{-5}$	282	10		22	253			
Zein,						270	81	20
Amino acid content, per cent.......	31.3 [6]	1.8 [6]	2.5 [6]	5.2 [3]	2.64 [3]			
Amino acid content gm. equi. $\times 10^{-5}$	213	13	15	29	189			
Casein,						265	157	155
Amino acid content, per cent.......	21.0 [7]	3.8 [9]	10.5 [7]	5.8 [3]	1.51 [3]			
Amino acid content gm. equi. $\times 10^{-5}$	143	26	64	32	108			
Fibrin,						105	24	85
Amino acid content, per cent.......	10.4 [8]	2.0 [8]		3.5 [8]	1.14 [3]			
Amino acid content gm. equi. $\times 10^{-5}$	71	15		19	81			

[1] Osborne and Guest (1911a).
[2] Abderhalden and Samuely (1905).
[3] This paper.
[4] Osborne and Clapp (1908).
[5] Kleinschmitt (1907).
[6] Dakin (1923).
[7] Dakin (1918).
[8] Abderhalden and Voitinovici (1907).
[9] Dunn and Lewis (1921).
[10] Cross and Swain (1924).
[11] Dakin (1919).
[12] Calculated by subtracting ammonia equivalents from total acidic equivalents.

determined with a fair degree of accuracy by present analytical methods.

The values for these groups together with other analytical data representing the chemical constitution of the present series of proteins are given in Table CVII. It is evident that the only relationships are between the amount of acid bound at pH 2.8 and pH 2.5 and the equivalents of free amino nitrogen of the native protein ($\varepsilon - NH_2$ of lysine); one-fourth of the arginine nitrogen, and the sum of these two nitrogen values. There is no relationship between the total acid or alkali bound and any of the nitrogen fractions as determined by the Van Slyke analysis.

In order to ascertain what relationship exists between (1) the free amino nitrogen of the protein or (2) one-fourth of the arginine nitrogen or (3) the sum of these values and the amount of acid bound at a given pH, the relationships were studied by statistical methods. Correlation coefficients were determined between the above factors and the acid binding capacity using the data given in Table CVIII. The formula as given by Harris (1910):

$$r = \frac{\Sigma(xy)/n - \overline{X}\overline{Y}}{\sqrt{(\Sigma(x^2)/n - \overline{X}^2)}\ \sqrt{(\Sigma(y^2)/n - \overline{Y}^2)}}$$

$$E_r = \pm \frac{0.6745\ (1 - r^2)}{\sqrt{n}}$$

was used for determining the correlation coefficient, *r*.

The values obtained are:

1. Correlation coefficient between gram equivalents of hydrochloric acid bound at pH 2.8 and gram equivalents of free amino nitrogen in the proteins, $r = 0.9923 \pm 0.00275$.
2. Correlation coefficient between gram equivalents of hydrochloric acid bound at pH 2.8 and gram equivalents of one-fourth of the arginine nitrogen, $r = 0.8651 \pm 0.04537$.
3. Correlation coefficient between gram equivalents of hydrochloric acid bound at pH 2.8 and gram equivalents of the free amino nitrogen plus one-fourth of the arginine nitrogen, $r = 0.9892 \pm 0.00388$.
4. Correlation coefficient between gram equivalents of hydrochloric acid bound at pH 2.5 and gram equivalents of one-fourth of the arginine nitrogen plus the free amino nitrogen, $r = 0.9918 \pm 0.00312$.

A valid correlation is obtained in all cases. The lowest correlation is obtained with one-fourth of the arginine nitrogen and the amount of acid bound at pH 2.8. The actual values used for correlation show a

TABLE CVII

SUMMATION OF ANALYSES CONCERNED WITH THE CHEMICAL BINDING

	Gram equiv. of N in 1 gram protein	Gram equiv. NaOH neut. to Thymol Blue	Equiv. NaOH bound at pH 10.2	Equiv. HCl bound at pH 2.8	Equiv. bound at maximum conc.		Equiv. HCl bound at pH 2.5	Equiv. of ½ lysine N (from Van Slyke)	Equiv. free NH_2—N	Equiv. of ¼ arginine N	Equiv. of NH_3—N	Equiv. of basic N	Equiv. ½ lysine + ¼ arg. N	Equiv. free NH_2—N + ¼ arg. N
					NaOH	HCl								
	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-4}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$
Gliadin	127.0	18.68	20	20	130	60	40	3.68	23.62	20.19	307	173	23.9	43.8
Speltin	121.6	20.71	20	22	130	70	40	18.85	19.09	19.94	297	184	38.8	39.0
Durumin	125.2	20.07	18	20	130	69	40	11.77	23.79	20.16	303	169	31.9	43.9
Dicoccumin	125.4	20.29	20	22	140	65	40	18.56	22.45	25.08	298	186	43.6	47.5
Monococcumin	119.1	20.71	20	20	135	65	40	13.70	20.25	21.44	286	187	35.1	41.7
Secalin	119.6	22.00	20	25	135	80	43	2.51	22.84	20.33	263	190	22.8	43.2
Sativin	107.7	32.00	27	15	145	65	35	6.43	17.77	24.02	236	153	30.5	41.8
Hordein	119.3	18.00	20	20	160	75	35	18.01	17.78	18.61	253	250	36.6	36.4
Zein	109.5	17.14	20	12	140	65	20	4.93	11.17	10.73	189	90	15.7	21.9
Teozein	113.6	16.14	17	15	140	65	28	14.65	17.04	11.13	210	123	25.8	28.2
Kafirin	112.5	15.71	17	10	170	60	25	13.95	14.18	11.03	233	105	25.0	25.2
Sorghumin	102.4	17.71	20	10	170	55	20	17.72	20.79	12.39	196	106	30.1	33.2
Casein	112.1	87.00	90	60	140	60	90	47.64	68.16	25.78	108	280	73.4	93.9
Fibrin	117.8	44.14	60	90	145	67	130	82.81	105.83	41.70	71	394	124.5	147.5

TABLE CVIII

DATA FROM WHICH THE CORRELATION COEFFICIENT r WAS CALCULATED

	Equiv. HCl bound at pH 2.8 I	Equiv. free NH_2—N II	Equiv. ¼ arginine N III	Sum of II and III IV	Equiv. HCl bound at pH 2.5 V	I-II	I-III	I-IV	V-II	V-III	V-IV
	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$						
Gliadin	20.0	23.62	20.19	43.81	40.0	—3.62	—0.19	—23.81	+16.38	+19.81	—3.81
Speltin	22.0	19.09	19.94	39.03	40.0	+2.91	+2.06	—17.03	+20.91	+20.06	+0.97
Durumin	20.0	23.79	20.16	43.95	40.0	—3.79	—0.16	—23.95	+16.21	+19.84	—3.95
Dicoccumin	22.0	22.45	25.08	47.53	40.0	—0.45	—3.08	—25.53	+17.55	+14.92	—7.53
Monococcumin	20.0	20.25	21.44	41.69	40.0	—0.25	—1.44	—21.69	+19.75	+18.56	—1.69
Secalin	25.0	22.84	20.33	43.17	43.0	+2.16	+4.67	—18.17	+20.16	+22.67	—0.17
Sativin	15.0	17.77	24.02	41.79	35.0	—2.77	—9.02	—26.79	+17.23	+10.98	—6.79
Hordein	20.0	17.78	18.61	36.39	35.0	+2.22	+1.39	—16.39	+17.22	+16.39	—1.39
Zein	12.0	11.17	10.73	21.90	20.0	+0.83	+1.27	—9.90	+8.83	+9.27	—1.90
Teozein	15.0	17.04	11.13	28.17	28.0	—2.04	+3.87	—13.17	+10.96	+16.87	—0.17
Kafirin	10.0	14.18	11.03	25.21	25.0	—4.18	—1.03	—15.21	+10.82	+13.97	—0.21
Sorghumin	10.0	20.79	12.39	33.18	20.0	—10.79	—2.39	—23.18	—0.79	+7.61	—13.18
Casein	60.0	68.16	25.78	93.94	90.0	—8.16	+34.22	—33.94	+21.84	+64.22	—3.94
Fibrin	90.0	105.83	41.70	147.53	130.0	—15.83	+48.30	—57.53	+24.17	+88.30	—17.53

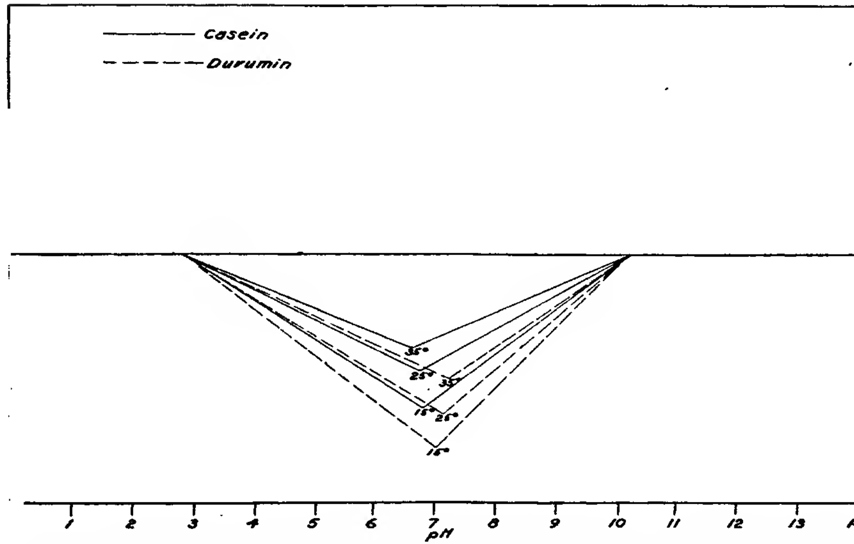


FIG. 19.—Theoretical curves drawn from mathematical calculations of the slope (constant b of the equation, log of acid bound $= a + b$ log of acid added) of the logarithmic curves for the equivalents of acid or alkali bound against the equivalents of acid or alkali added where the lines are made to intersect at pH 2.5 and pH 10.5. (See text for explanation.)

close relationship in only two, (1 and 4) of the four cases (cf. Table CVIII). When the amount of free amino nitrogen and the amount of acid bound at pH 2.8 are compared, not only is a perfect correlation obtained but the actual experimental and the calculated values check very closely. Also, when the amount of acid bound at pH 2.5 and the sum of the free amino nitrogen plus one-fourth of the arginine nitrogen are compared, a very good correlation is obtained and the actual values (experimental and calculated) check very well in most cases. These calculations support the theory that the chemical combination taking place between the isoelectric point of the protein (no acid or alkali bound) and pH 2.5 is due to the combination of acid with the free amino nitrogen of the native protein which is neutralized at about pH 2.8 and the sum of the free amino nitrogen and one-fourth of the arginine nitrogen which is neutralized at about pH 2.5.

Isoelectric Point. The possibility of calculating the isoelectric point of proteins has been studied by several investigators but no satisfactory method has been worked out. It has been suggested that the point on the buffer curve where neither acid nor alkali is bound by the protein is the isoelectric point. It is almost impossible to determine this point, due to the wide "isoelectric range." No definite point where the curve crosses the zero line can be assigned.

The logarithms of the equivalents of acid or alkali bound or the logarithms of the original acid or alkali concentration as ordinates plotted against the final pH as abscissa give a straight line. The constants a and b for the straight line formula were calculated from the data where the hydrogen ion concentration is greater than pH 2.5 and the hydroxyl ion concentration greater than pH 10.5.

The constants a and b of the logarithms of the equivalents of acid and alkali bound plotted against the final pH, as shown in Tables CI and CV, were used to plot the theoretical curves, Fig. 19, and *the point of intersection is considered as the isoelectric point.* The values for this point in terms of pH are given in column II, Table CIX.

As shown in Figs. 12 to 15 all of the lines pass through a common point at about pH 2.5 for acid and at about pH 10.5 for alkali. Apparently this holds true for all proteins. Lines were accordingly drawn through these points using the values for b as given in Table C, as the tangent of the angles. Such curves are illustrated in Fig. 20, where casein and durumin are selected as examples. The intersection of the lines should, from theoretical considerations, give the isoelectric point, *if we assume that this exterpolation can be correctly made.* These values in terms of pH are given in column III, Table CIX.

When the logarithms of the acid or alkali added are plotted against the final pH, straight lines are likewise obtained. The constants a and b were calculated from this data for all of the proteins and are given in Table CX. From these values, the lines were plotted. The signs

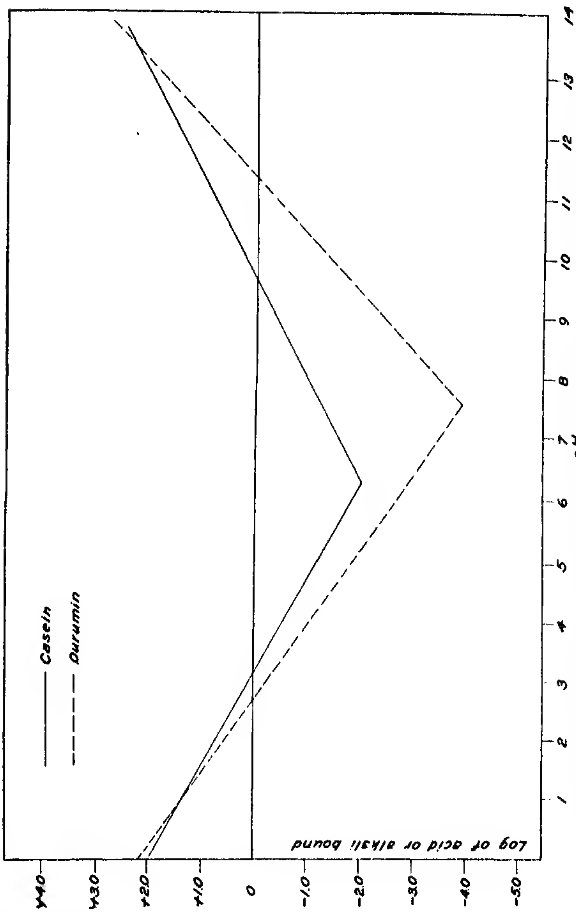


FIG. 20.—Theoretical curves drawn from the mathematical formula, log of acid bound $= a + b$ log of acid added, where both constants are used. The point of intersection is the theoretical iso-electric point as determined from this data.

for the constants for alkali plus protein were changed from minus to plus in order to bring the lines in adjacent quadrants on the graph. Such a change did not affect the conclusions relative to the position of the isoelectric point. These values are given in column IV of Table CIX. The point of the intersection was taken as the isoelectric point. In these calculations of the constants *a* and *b*, all 19 points (from the experimental data) were used for each protein. With the exception

TABLE CIX

ISOELECTRIC POINT OF THE VARIOUS PROTEINS, IN TERMS OF pH

	I Determined potentiometrically [18]	II Calculated from data in tables 101 and 105	III Calculated from data in tables 100 and 104	IV Calculated from data in table 110
Gliadin	5.76	7.60	6.97	7.16
Speltin	4.60	7.54	6.90	7.00
Durumin	5.98	7.55	7.00	7.31
Dicoccumin	5.45	7.47	7.01	7.08
Monococcumin	4.53	7.72	6.95	7.33
Secalin	5.43	7.32	6.40	7.10
Sativin	4.40	6.60	6.96	7.12
Hordein	5.18	7.40	6.36	6.90
Zein	5.00	6.53	6.60	6.73
Teozein	6.45	6.30	6.51	6.55
Kafirin	4.24	6.95	6.50	7.03
Sorghumin	4.18	6.40	6.40	7.08
Casein	4.77	6.31	6.72	5.10
Fibrin	4.80	6.20	6.45	6.70
Durumin 15°		7.39	6.70	7.70
Durumin 25°		7.62	6.92	7.71
Durumin 35°		8.00	7.12	7.83
Teozein 15°		6.95	6.80	7.00
Teozein 25°		7.12	6.71	6.80
Teozein 35°		7.16	6.52	6.89
Casein 15°		6.73	6.67	5.15
Casein 25°		6.76	6.40	5.10
Casein 35°		6.80	6.42	5.30
Fibrin 15°		6.88	6.96	6.85
Fibrin 25°		7.00	7.00	6.67
Fibrin 35°		6.53	6.78	6.80

of the first four points of the casein curve, all points lay very near the theoretical line.

The hydrogen ion concentrations of a one per cent protein suspension in pure water as pH determined potentiometrically are given in column I, Table CIX. It is noted that in all cases except teozein, the measured hydrogen ion concentration is greater than the calculated value. The values calculated for the various proteins with the exception of casein show a remarkable agreement but they do not agree with such values as are found in the literature. The experimentally determined values (column I) are in much closer agreement with the published data.

[18] Hydrogen ion concentration of a 1% suspension of the protein in pure water.

From the data of Tables LXXVIII to XCIII, it is readily seen why the logarithmic curves used to calculate the isoelectric points cannot be exterpolated to give the true isoelectric point. If, however, we consider that there are two types of acid or alkali binding, one of a chemical nature and the other physical adsorption, then *the values given in Table CIX, Columns II, III and IV are the isoelectric points as determined from the adsorption type of binding or are the isoelectric points for the binding of acid above a hydrogen ion concentration of pH 2.5 and the binding of alkali above a hydroxyl ion concentration of pH 10.5.*

TABLE CX

CONSTANTS FOR THE LOGARITHMIC CURVE, $y = a + bx$, FOR THE ACID OR ALKALI BINDING OF VARIOUS PROTEINS, WHERE y = THE LOG OF EQUIVALENTS OF ACID OR ALKALI ADDED AND x = THE pH OF THE EQUILIBRIUM SOLUTION

	Hydrochloric acid		Sodium hydroxide [17]	
	a	b	a	b
Gliadin	3.0491	−0.8929	10.5124	−0.9872
Speltin	3.0612	−0.9200	10.1550	−0.9603
Durumin	2.9516	−0.8928	11.3565	−1.0534
Dicoccumin	3.1348	−0.9630	11.4465	−1.0607
Monococcumin	3.0401	−0.8795	11.2930	−1.0486
Secalin	3.0852	−0.8913	10.3864	−0.9586
Sativin	3.1979	−1.0353	10.6707	−1.0017
Hordein	3.0704	−0.9024	10.2160	−0.9689
Zein	3.2514	−1.0214	10.4871	−0.9885
Teozein	3.3434	−1.0999	10.0567	−0.9885
Kafirin	3.1822	−1.0303	11.6991	−1.0858
Sorghumin	3.1790	−1.0993	12.3295	−1.1411
Casein	2.9288	−0.7768	3.5576	−0.4490
Fibrin	2.9478	−0.7352	5.1695	−0.5735
Durumin 15°	3.1604	−0.9844	11.3027	−1.0515
Durumin 25°	2.9286	−0.8679	10.8253	−1.0101
Durumin 35°	2.9010	−0.8543	10.9599	−1.0163
Teozein 15°	3.2871	−1.0712	10.4795	−0.9894
Teozein 25°	3.3383	−1.1083	8.4781	−0.8308
Teozein 35°	3.0093	−0.9653	10.0444	−0.9483
Casein 15°	2.9962	−0.8381	4.4796	−0.5221
Casein 25°	2.9165	−0.8174	3.3994	−0.4351
Casein 35°	2.8297	−0.7651	3.3784	−0.4312
Fibrin 15°	2.9199	−0.7416	6.2822	−0.6641
Fibrin 25°	2.8092	−0.7027	5.9679	−0.6358
Fibrin 35°	2.7680	−0.6652	5.6856	−0.6103

The values given in Column I, Table CIX, are essentially the same as those indicated by the buffer curve of the dilute alkali or acid plus protein. Here the "isoelectric point" is not definite, *i.e.*, a definite pH value, but covers a wide "isoelectric range" such as has been noted by Michaelis (1914), Bayliss (1923) and Levene and Simms (1923). In the case of fibrin, this isoelectric range is quite narrow lying between

[17] Signs for *a* and *b* changed, see text, page 352.

about pH 4.0 and pH 5.5 and for casein it is between about pH 4.0 and pH 6.0. Durumin and teozein show a much wider isoelectric range, durumin being from about pH 5.0 to pH 9.0 and teozein from about pH 4.5 to pH 9.5. The isoelectric point of the chemical phase of this combination or *the true isoelectric* point of the protein must be somewhere near the measured value as determined potentiometrically or, in other words, the isoelectric point of a protein is determined by the ionization of acidic and basic groups within the molecule. If the acidic groups predominate the isoelectric point will lie on the acid side of pH 7 and if the basic groups predominate it will be on the alkaline side. In proteins of which teozein appears to be an example, where acidic and basic groups tend to neutralize each other the isoelectric point will be in the neighborhood of neutrality (pH 7). The distance that the isoelectric point is removed in either direction from neutrality apparently determines to a considerable extent the amount of acid or alkali which will be bound between hydrogen ion concentrations represented by pH 2.5 and 10.5. In any event, however, this *chemical isoelectric point* tells us nothing concerning the amount of acid or alkali bound on the acid side of pH 2.5 or on the alkaline side of pH 10.5. *In these regions where physical adsorption appears to be the predominating if not the sole factor, all proteins tested regardless of their chemical combination or biological source bound essentially the same amount of acid or alkali per gram of protein at any given pH* (outside the region between pH 2.5 and pH 10.5).

The chemical nature of a protein and the power of a protein to bind acid and alkali in stoichiometrical relationships depends upon the chemical groups within the protein molecule and is therefore limited to the range between pH 2.5 and pH 10.5. Thus our findings afford a logical explanation for the divergent views of Loeb *et al.* and other workers who hold that acid and alkali binding are of a stoichiometrical chemical nature and those workers who insist that colloidal adsorption is the predominating factor. *Both are correct,* and we have shown in what regions (in terms of hydrogen ion concentration) one or the other phenomenon may be expected to predominate.

V. SUMMARY AND CONCLUSIONS

The known alcohol soluble proteins from wheat, *Triticum vulgare,* spelt, *Triticum spelta,* rye, *Secale cereale,* oats, *Avena sativa,* barley, *Hordeum vulgare,* corn, *Zea mays,* and kafir, *Andropogon sorghum,* were prepared and analyzed for their nitrogen and sulfur content. The unknown alcohol soluble proteins from durum, *Triticum durum,* emmer, *Triticum dicoccum,* einkorn, *Triticum monococcum,* teosinte, *Euchlaena mexicana Schrad.* and sorghum, *Sorghum vulgare,* were prepared and

analyzed. Casein and fibrin were also prepared and analyzed. The data on the elementary analysis do not show any striking differences between the various proteins.

The nitrogen distribution, the free amino nitrogen, the free carboxyl groups, the true ammonia nitrogen and the tryptophane and cystine content of this series of proteins were studied. A close similarity was noted between the proteins of a single group of cereals (*i.e.*, the wheat group or the maize group) but there is a distinct difference between the proteins prepared from different types of cereals. Although certain differences in chemical analyses were found between the proteins prepared from a single type of cereals, these differences were not sufficiently marked to enable one to sharply divide the cereal prolamines into sub-classes.

The binding of acid and alkali by the various proteins was studied. Because of its recognized accuracy the potentiometric method was used almost exclusively. All of the proteins, regardless of their chemical composition, bound, gram for gram, approximately the same amount of acid or alkali, when the final hydrogen ion concentration was greater than pH 2.5 and the hydroxyl ion concentration was greater than pH 10.5. The logarithms of the equivalents of acid or alkali bound by the proteins when plotted as ordinates against the logarithms of the equivalents of acid or alkali added, or the final pH as abscissa gave straight lines. The constants *a* and *b* for the formula for a straight line were calculated for all such curves resulting from the experiments with the present series of 14 proteins. The values for the constants are almost identical for the proteins prepared from the same type of cereals.

Approximately equivalent amounts of hydrochloric, sulfuric and phosphoric (molar) acid were bound by a unit amount of protein, when the acids are compared on the normality basis. Equal amounts were not bound at the same equilibrium hydrogen ion concentration as claimed by Loeb, much more phosphoric acid than hydrochloric acid being bound.

A negative temperature coefficient was obtained when the experiments on the binding of hydrochloric acid and sodium hydroxide were carried out at 15°, 25°, and 35° C. and when the final hydrogen ion concentration was more than pH 2.5 and the hydroxyl ion concentration was more than pH 10.5. The ratio was approximately 1:2:3 where the amount bound at 35° is 1. When the logarithms of the equivalents of acid or alkali bound at the different temperatures were plotted against the logarithms of the equivalents of acid or alkali added, the lines for a single protein passed through common points. For acid this point represented a hydrogen ion concentration of about pH 2.5 and for alkali, a hydroxyl ion concentration of about pH 10.5.

Experiments were carried out where more dilute acid and alkali

were used, in an attempt to determine the behavior of acid and alkali binding between the hydrogen ion concentrations represented by pH 2.5 and pH 10.5. Here the amount of acid or alkali bound apparently depends on the chemical composition of the protein. The buffer curve does not form a smooth line. When the logarithms of the equivalents of acid or alkali bound in this pH region are plotted against the final pH, the curves do not form a straight line. It is suggested that there are two types of combinations between proteins and acid or alkali: (1) a chemical type of combination which takes place between a hydrogen ion concentration represented by pH 2.5 and pH 10.5 and (2) an adsorption type of combination which takes place when the hydrogen ion concentration is greater than pH 2.5 or the hydroxyl ion concentration is greater than pH 10.5.

Evidence of a chemical type of combination between a hydrogen ion concentration of pH 2.5 and pH 10.5 is presented by:

1. The logarithms of the amount of acid or alkali bound plotted against the original concentrations do not form a straight line.
2. The buffer curves do not form a smooth, regular line.
3. The amount of acid or alkali bound at any hydrogen ion concentration between pH 2.5 and pH 10.5, depends on the chemical composition of the protein. This is not true where the pH is less than 2.5 or greater than 10.5.
4. When the hydrogen ion concentration is below about pH 2.5, the protein chloride is highly ionized. (*Cf.* Pauli, and Hitchcock.)

Evidence of the adsorption type of combination is furnished by:

1. At the higher concentrations of acid and alkali, all of the proteins used in this work *regardless of their chemical composition,* bind approximately the same amount of acid or of alkali.
2. There is a marked negative temperature coefficient of the acid or alkali binding at the higher concentrations of acid and alkali.
3. The logarithms of the amount of acid or alkali bound plotted against the logarithms of the original acid or alkali concentration or against the final pH form a straight line.
4. There is more alkali bound when the original concentration is 0.500 normal than can be accounted for by chemical combination assuming that there is an available carboxyl group for each nitrogen atom, an assumption far in excess of possibility.
5. When the hydrogen ion concentration is greater than about pH 2.5 there is no increase in the ionization of the protein chloride. (*Cf.* Pauli, and Hitchcock.)

The analytical data in regard to the amino acid content of the prolamines are not sufficiently accurate to enable final conclusions to be drawn as to the chemical groups responsible for the chemical binding of alkali. In the case of acid binding, however, a correlation of $r = 0.9923 \pm 0.00275$ was found between the free amino nitrogen of the protein as determined in the Van Slyke apparatus and the equivalents of acid bound at pH 2.8, and a correlation of 0.9918 ± 0.00312 was obtained between the sum of the free amino nitrogen plus one-fourth of the arginine nitrogen (the free amino group of the guanidine nucleus) and the equivalents of acid bound at pH 2.5. As already noted the character of acid binding changes at pH 2.5.

If the isoelectric points are calculated by exterpolating the logarithmic curves of the second type, the acid and alkali curves intersect in the neighborhood of pH 7, the neutral point as referred to water. This is the case when the isoelectric point was calculated from, (1) the logarithms of the amount of acid or alkali bound by the proteins and the logarithms of the original concentration of acid or alkali, (2) the logarithms of the amount of acid or alkali bound and the equilibrium pH and (3) the logarithms of the amounts of acid or alkali added and the equilibrium pH. The isoelectric point of the protein, *i.e.*, the hydrogen ion concentration of the protein suspended in water, determined potentiometrically does not in a number of instances agree with these exterpolated values but is found to be in the neighborhood of the values reported in the literature as determined directly by cataphoresis or other methods. The measured isoelectric "point" of a protein probably is not a definite point but should in all probability be referred to as an "isoelectric range." *The position of this isoelectric range on the pH scale is dependent on the chemical composition of the protein.* The calculated isoelectric point is very near the hydrogen ion concentration of neutral water. This is what would be predicted on the theory that at the higher concentrations of acid and alkali, the binding of acid and alkali follow the adsorption law. *The calculated isoelectric points are not related to the chemical composition of the protein.*

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